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- 9 JAN 2004

The Patent Office
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1. Your reference GBP89326

2. Patent application number
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3. Full name, address and postcode of the or of each applicant (underline all surnames)

Isis Innovation Limited,
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Oxford
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United Kingdom

03998564003
Patents ADP number (if you know it)

09 JAN 2004

0400438.8

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention Improved Vaccines

5. Name of your agent (if you have one)
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Marks & Clerk
57 - 60 Lincoln's Inn Fields
London WC2A 3LS

Patents ADP number (if you know it)

18001

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Country	Priority application No (if you know it)	Date of filing (day / month / year)

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8. Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?

Yes

(Answer 'Yes' if:
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Patents Form 1/77

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form	0
Description	75
Claim(s)	4
Abstract	1
Drawing(s)	

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10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	
Request for preliminary examination and search (Patents Form 9/77)	
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11. I/We request the grant of a patent on the basis of this application.

Signature(s) Marks & Clerk Date: 9 January 2004

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Patent Chemical Formalities
020 7400 3000

IMPROVED VACCINES

The present invention provides novel methods of vaccination.

Vaccination is a useful tool for combating and preventing disease caused by a number of agents. Such agents may be exogenous, such as viruses, bacteria or parasites. Alternatively, disease-causing agents may be endogenous, such as tumours. Immune responses induced by vaccination may be divided into humoral or antibody responses, and cellular immune responses, such as those mediated by T lymphocytes. A large number of vaccines act by generating protective levels of antibodies.

Antibody-inducing vaccines frequently employ either an entire microorganism, that is often attenuated or heat-killed, or a sub-unit component of the microorganism with an adjuvant. Favoured adjuvants for inducing strong antibody responses include Alum, NP59, CpGs, AS02 and various emulsions.

However, it has recently become clear that cellular immune responses may also be of some use on their own. Various means have been used to induce strong protective cellular immune responses by vaccination.

Known means of generating strong cellular immune responses include DNA vaccination, immunisation with viral vectors or protein particles, the use of recombinant bacteria such as BCG, and heterologous prime-boost immunisation. In fact, heterologous prime-boost immunisation approaches have been found to induce particularly strong effector T cell responses in animals and humans. Such regimes may comprise priming with DNA followed by boosting with recombinant modified virus Ankara (MVA), or priming with recombinant fowl pox and then boosting with recombinant MVA.

In order to combat these more effectively, it is desirable to induce stronger immune responses. However, vaccination methods that generate high level antibody responses differ significantly from those that engender strong cell-mediated or T cell responses. For example, Alum is a useful adjuvant for inducing antibodies but

generates weak or negligible $CD8^+$ T cell responses. In contrast, heterologous prime-boost immunisation methods have induced strong T cell responses in humans, but only minimal antibody responses. However, immune protection against many diseases can be mediated by either T cells or antibodies at sufficient levels, and optimal protection may be achieved by inducing strong responses of both types. For example, in malaria, it is thought that a pre-erythrocytic vaccine capable of inducing of both high level anti-sporozoite antibodies as well as a strong T cell response against the liver stage parasite would be an ideal method of preventing or treating the disease. However, no vaccination approach currently exists that allows strong responses of each type to be generated. This problem exists not only in malaria, but in a large number of other diseases.

Accordingly, there is still a need in the art for a method of vaccination that can not only induce a high level antibody response, but also a strong cellular or T cell mediated response. The present invention sets out to overcome this need by providing novel vaccination methods capable of inducing both strong antibody and T cell responses.

Surprisingly, the present inventors have found that co-administration of an antigen with a viral vector induces both strong antibody and cell-mediated immune responses in a target mammal.

According to one aspect of the present invention, there is provided a vaccine for inducing an immune response to an antigen, the vaccine comprising the antigen and a vector, the vector in the absence of the antigen inducing a weak or negligible antibody response and in the presence of the antigen inducing a T cell response complementing the antibody response against said antigen, the vector and the antigen being formulated separately or together.

Preferably, the vector and the antigen are formulated for co-administration.

It is preferred that the vaccine is suitable for administration in a homologous prime boost vaccination regimen or, alternatively, a heterologous prime boost vaccination regimen.

Preferably, the antigen and the viral vector are admixed and administered as a mixture, as discussed below.

The nature of the viral vector is also discussed below. It is preferred, however, that the viral vector is a poxvirus or an adenovirus. Preferably, the vector is MVA, NYVAC, ALVAC or a fowlpox virus. However, in one embodiment of the present invention it is preferred that the vector is not ALVAC.

It is preferred, as also discussed below, that the antigen is derived from *M. tuberculosis*, *Plasmodium sp,* influenza virus, HIV, Hepatitis C virus, Cytomegalovirus, Human papilloma virus, bacteria, *Plasmodium sp,* leishmania parasites or is derived from a tumour. Preferably, the bacteria are Mycobacteria.

Also provided is a vaccine that induces both an effector T cell response and an antibody response, wherein the effector T cell response is not weaker than that induced by the viral vector alone, and the levels of antibody induced are not lower than those induced by administration of the antigen alone.

The present invention also provides a method for stimulating both humoral and antibody responses to an antigen, comprising administration of the antigen to a patient in combination with a viral vector, administration of the vector and antigen being separately or together.

It is preferred that the antibody response to the co-administered antigen is greater than the antibody response induced by the administration of a vaccine comprising said antigen and alum, but without the vector.

It is also preferred that the T cell response induced by the vaccine is epitope-specific.

Also provided is a vaccine, wherein the poxviral vector is an orthopox virus, such as MVA or NYVAC, the presence of an orthopox viral vector inducing substantially equal ratios of Th-1 and Th-2 Helper T cells.

Alternatively, the poxviral vector is an avipox virus, such as Fowlpox or Canarypox, the presence of an avipox viral vector inducing a T cell mediated response, wherein the Th-1 Helper T cell response is greater than the Th-2 response.

There is also provided a vaccine, comprising a polynucleotide and a co-administered antigen, the polynucleotide inducing a T cell response and the co-administered antigen inducing an antibody response, both the T cell and antibody responses being directed to said antigen;

wherein administration of the vaccine comprising the polynucleotide but not the co-administered antigen induces a weak or negligible antibody response;

the polynucleotide and the co-administered antigen being formulated separately or together, being further formulated for co-administration,

the Th-2 Helper T cell-mediated response being greater than the Th-1 response.

It can be seen that in this manner, Th-1 or Th-2 biased responses can be achieved for a particular antigen, so as to enable the administrator of the vaccine to, at least partially, select which, or both, of the Th-1 and/or Th-2 responses to enhance for a particular antigen. The skilled person would be able to determine which of these (Th-1 or Th-2 biased, or both) responses would be most appropriate for the antigen vaccination regimen that he is administering.

The present inventors have shown that prime-boost immunisation with DNA and MVA (D/M) induces potent T-cell responses but poor levels of antibody against the encoded HBsAg. Conversely, repeat immunisation with Engerix-B induced a weak T cell response but greatly increased antibodies to HBsAg compared to D/M.

Surprisingly, however, the present inventors have found that concurrent administration of these vaccines induced both T cells and antibodies to HBsAg (Hepatitis B surface Antigen). Further improvements of this co-induction of cell-

mediated immunity (CMI) and humoral immunity were sought by combining the viral vectors MVA (Modified Vaccinia Ankara), FP (Fowlpox), ALVAC (avipox virus), NYVAC (attenuated vaccinia virus) and ADV (adenovirus) with the main component of the commercially available Engerix-B vaccine, HBsAg.

So called "non-recombinant" vectors were used which did not encode an antigenic peptide, but did encode a non-antigenic detectable marker, for instance the *LacZ* gene.

It was found that non-recombinant MVA, FP, ALVAC and NYVAC vectors adjuvanted co-administered protein leading to greatly enhanced levels of T cells and antibodies against the protein.

These results are also supported by experiments in a second model. Vaccines strategies for malaria are often initially tested in the murine model, *P berghei*. Two of these vaccines include the T cell inducing vectors FP and MVA, both encoding the *P. berghei* circumsporozoite protein (FP.CSP and MVA.CSP), and an antibody inducing vaccine, Apv, consisting of Hepatitis B core (HBc) protein containing two copies of the *P. berghei* CSP B cell epitopes (DP_4NPN) adsorbed to alum. Prime-boost immunization with FP.CSP and MVA.CSP induced potent $CD8^+$ T cells and no detectable antibodies to the B cell epitope DP_4NPN. Conversely, repeat immunization with Apv induced strong levels of antibodies to DP_4NPN.

Surprisingly, co-administration of FP.CSP, MVA.CSP and Apv lead to specific induction of potent antibodies to both DP_4NPN, and high T cells cell responses to a $CD8^+$ epitope in CSP and HBc. Thus, the present inventors show in two different systems that viral vectors, for instance poxviruses, can enhance both T cell and antibody responses to protein thereby demonstrating a method of strongly inducing both cellular and humoral types of immunity against a target antigen.

The dose of poxvirus to be used according to the present invention will be typically in the range of 10^5 to 10^7 pfu in small animals such as mice and in the range of 10^7 to 10^{10} pfu in large animals, such as primates and ruminants, including humans. As

individual antigens may vary in immunogenicity it may be necessary to undertake dose escalation studies with increasing doses of poxvirus to find the optimal dose. Such dose finding studies are familiar to those skilled in the art.

The present inventors have found that co-administration of an antigen with a viral vector induces both strong antibody and cell-mediated immune responses in a target mammal.

The antigen and the viral vector may be administered together or separately, and may be administered at the same time or over a period of time, preferably on the same day, particularly within 2 to 3 hours, and more preferably substantially together.

Administration of the antigen with the viral vector surprisingly stimulates both a humoral and an antibody response to the antigen, thereby providing an immune response on both levels.

The nature of the viral vector is not critical to the present invention. In general the vector should be able to stimulate a T cell response. Suitable examples of viruses are provided below. It is preferred that the viral vector is incapable of causing a serious infection in the patient, and it is generally preferred that the virus is incapacitated, such as by heat treatment or attenuation. Empty capsids may be employed.

The present invention further provides vaccines comprising both the antigen and viral vector of the invention, as well as kits comprising preparations thereof.

Preferably, the antibody levels induced in the target mammal are greater in the vaccination method according to the present invention than those seen on administration of the antigen alone. Furthermore, it is also preferred that the T cell response induced as a result of the vaccination method according to the present invention is not less than an order of magnitude less than that induced by administration of the viral vector alone. Preferably, the T cell response induced by the present invention is not decreased, and more preferably is increased, compared to the T cell response elicited by the viral vector alone. Most preferably, both the levels of antibody and the T cell response induced by the present invention are greater than that achieved by administration of the antigen or the viral vector alone.

Preferably the antigen is proteinaceous. Alternatively, it is also preferred that the antigen is a peptide. Furthermore, it is preferred that the viral vector is attenuated, heat-killed or unable to replicate. Whilst it is envisaged that the viral vector is derived from any virus known in the art that is suitable for use therefor, it is particularly preferred that the viral vector is selected from the group consisting of retroviruses, adenoviruses and adeno-associated viruses. More preferably, the viral vector is derived from the herpes viridae family, preferably the varicella viruses, or from the pox viridae family, preferably MVA or fowl pox.

The antigen and the viral vector are preferably administered within several days of each other but more preferably within 3 hours and most preferably within one hour of each other. Alternatively, the antigen and the viral vector may be administered at the same time, either separately, or more preferably, as a mixture.

Administration of the antigen and the viral vector, whether independently or as a mixture, may be orally or transdermally, but most preferably parenterally. The site of parenteral administration may be intravenously, intramuscularly, subcutaneously, or intradermally, or by any other means known to the skilled person.

The vector may, optionally, comprise a nucleic acid encoding a protein or peptide. The expressed product of this nucleic acid is preferably an antigen. This expressed antigen may be homologous to the antigen administered with the viral vector, but may also be heterologous. Preferably, the expressed protein or peptide is recombinant.

The subject or patient to which the vaccine is administered is preferably an animal, more preferably a mammal, even more preferably a primate and it is particularly preferred that the patient is a human.

The antigen may be derived from a virus, such as HIV or the Hepatitis B virus, preferably the epitope is derived from a coat or backbone protein. In a preferred embodiment, the antigen comprises the Hepatitis B surface Antigen (HbsAg). However, it is also preferred that the antigen is derived from a parasite, such as a

malarial parasite from the *Plasmodium* family, a bacteria, such as *M. tuberculosis*, or may even be endogenously derived, for instance a tumour antigen.

The vector may comprise no antigen-encoding nucleic acid. Accordingly, it is preferred that the vector comprises a nucleic acid encoding a marker such as Lac Z, but no antigenic protein or peptide. Preferably, the vector is an empty vector.

In combating HIV, neutralization or opsonisation of HIV-1 by antibodies combined with the action of CTL suppression of viral replication and induction of cell death of virally infected cells, may have a multiplicative effect on R_o, the mean number of cells that are infected by a single infected cell. By reducing R_o, the spread of virus and, therefore, viral load, may be limited. This may allow the immune system to focus on rapidly controlling initial infection. Limiting the amount of viral replication may also reduce the virus' opportunity to mutate following immune pressure of specific epitopes which may, therefore, limit viral escape.

The method of vaccination according to the present invention may simply comprise administration of the antigen and the viral vector. Alternatively, the method may comprise administration of other elements such as adjuvants, such as Alum, or other vaccines. The method may also be part of a more complex vaccination regimen, for instance that comprising a homologous, but preferably a heterologous, prime-boost vaccination regimen.

Therefore, although the present invention may be used in isolation, it may also be combined with other vaccination regimens for combating or preventing the same disease. Alternatively, it may be part of a regimen for combating or preventing more than one disease. Accordingly, the present invention may be used in a regimen that elicits an immune response to more than one antigen. For instance, the viral vector may comprise a nucleic acid that encodes a protein or peptide derived from HIV, whilst the antigen administered according to the present invention is derived from *M. tuberculosis*. Therefore, in another embodiment of the invention, the viral vector may encode a protein or peptide that is derived from a different disease-causing agent than the antigen co-temporaneously administered with the vector.

The T cell response induced is preferably a T cell response. Preferably, the response comprises a $CD4^+$ T Helper cell response, or a $CD8^+$ Cytotoxic T Lymphocytes (CTL) response, and most preferably both, such as a CTL response mediated by T Helper cells, for instance.

WO 00/00216 (Pasteur Meriux Serums et Vaccins) discloses that the use of a poxvirus, such as Canarypox (ALVAC), in combination with an antigenic peptide, for example an HIV-derived peptide, provides an enhanced antibody response to the antigenic peptide. However, all of the experiments therein relate solely to measuring antibody responses and there is no demonstration of an increased cell-mediated or T cell response.

Clearly, an increased antibody response does not occur spontaneously and *in vacuo*, but rather is regulated to some extent, depending on the type of antibodies raised, by certain T cell types. However, as is shown in the examples, it is not sufficient to simply measure antibody isotypes to demonstrate that cellular immunity has been induced. Although there is a correlation between Ab and T cell responses, it is weak and inconsistent, such that in many immune responses, one simply cannot be predicted from the other.

However, we have shown that cellular responses can be generated to an antigen encoded by a recombinant viral vector encoding the antigen, and that it is this encoded antigen that is generating the CD4 and CD8 T cell responses. However, the putative cellular immunity referred to in WO 00/00216 relates to the co-administered antigen (i.e. the protein given with the poxvirus). However, we have shown that the cellular response to this may be weak or even absent when the poxvirus does not encode the same antigen.

The present inventors have also shown that poxvirus can induce T cell responses at the same time as enhancing antibody responses to the co-administered antigen, the levels of T cell induction being surprisingly high.

The inventors have also shown that orthopoxviruses, such as MVA and NYVAC, and fowlpox are useful in adjuvanting antibody induction to a co-administered antigen.

Accordingly, there is provided a vaccine that induces both an antigen-specific effector T cell response to a virally-encoded antigen and also induces an antibody response to a co-administered antigen.

In the case that the vector does comprise a polynucleotide encoding an antigen, it is preferred that the T cell response is to the vector-encoded antigen, rather than the co-administered antigen.

It is also preferred that the vector, regardless of whether the vector comprises a polynucleotide encoding an antigen or does not comprise such a polynucleotide, can be used as an adjuvant to enhance the levels of antibodies induced to the co-administered antigen.

Preferably, the virally -encoded antigen is heterologous to the virus and may, as discussed above, originate from a disease-causing agent, such as HIV, a pathogen or a tumour.

It is also preferred that the virally -encoded antigen is a polypeptide, and it is further preferred that said antigen comprises a source of $CD4^+$ and $CD8^+$ epitopes.

Preferably, the co-administered antigen is not a nucleic acid and may, therefore, preferably also be a polypeptide.

Furthermore, it is preferred that the vector-encoded antigen and the co-administered antigen are homologous, thereby providing a strong immune response on both cellular and humoral levels.

However, it is also envisaged that that they may be heterologous, such that they may be derived from different disease causing agents, for instance, different pathogens,

or one from a pathogen and one from a tumour. The advantage of this approach is that the immune response will be to more than one antigen, thereby allowing for the present invention to be used in so called "combination vaccines".

The viral vector used may be adenovirus. However, it is preferred that it is a poxviral vector. Preferably, the poxvirus is an orthopox virus for instance such MVA or NYVAC, or an avipox, virus for instance fowlpox, ALVAC, the FP9 avipox virus, or derivatives thereof. It is also preferred that that vector is replication-impaired.

The vector and the antigen are preferably formulated for co-administration, regardless of whether they have been formulated separately or together. Preferably they are co-administered as a mixture.

The method of administration is, preferably, may be intradermally, intravenously, intraperitonealy, intramuscularly, orally, intranasally, by aerosol or subcutaneously.

It is preferred that the vaccine is used to amplify a pre-existing antibody or T cell response, preferably generated by a means other than a prior priming immunization with the same vaccine or vaccines.

Pathogens that may provide suitable antigens are: Hepatitis B virus, influenza virus, HIV, Hepatitis C virus, Cytomegalovirus, Human papilloma virus, Mycobacteria, other bacteria, *Plasmodium sp,* leishmania parasites. Preferably, the co-administered antigen is particulate.

It is preferred that the induced T cell response is a $CD8^+$ T cell response. However, it is also preferred that the T cell response is a $CD4^+$ T cell response. Preferably, the immune responses induced are protective.

Preferably, the induced antibody responses to the co-administered antigen are of a greater magnitude than those induced by the same antigen adjuvanted by alum. Furthermore, it is also preferred that the T cell response induced to the co-administered antigen is of greater magnitude than in the absence of the viral vector.

It is envisaged that the antigen and/or vector may be mixed or co-formulated with alum or other known adjuvants.

It is also preferred that the virally-encoded antigen comprises a CD4+ and / or CD8+ T cell epitope against which the vaccinee has a pre-existing specific cellular immune response. Preferably, the immune response was generated by a means other than by immunization with the said recombinant virus, i.e. in a heterologous prime boost regimen.

Also provided is a vaccination method comprising co-administering an antigen together with a vector,
the method inducing both
- an antigen-specific T cell response to a poxvirus-encoded antigen, the encoded antigen being heterologous to the poxvirus and comprising a source of $CD4^+$ and $CD8^+$ epitopes; and
- antibodies to the co-administered antigen.

Preferably, the co-administered antigen is not a polynucleic acid.

Also provided is a method of inducing antigen-specific T cell responses in a vertebrate to a poxvirus-encoded heterologous polypeptide antigen comprising a source of $CD4^+$ and $CD8^+$ epitopes for the vaccinee and inducing antibodies to a co-administered (non-encoded) non-nucleic acid antigen by co-administration of the non-encoded antigen mixed with the said poxvirus.

Further provided is a method of generating an antibody response to an antigen in a vertebrate vaccinee by co-administration, as a mixture, the antigen mixed with an orthopox virus.

Preferably, the orthopox virus is replication-impaired. It is also preferred that the orthopox virus is of the modified vaccinia virus Ankara strain or NYVAC strain or a derivative of either. Preferably, the orthopox virus encodes the co-administered antigen

or a homologous sequence. Alternatively, the orthopox virus may encode an antigen that is heterologous to the co-administered antigen.

It is preferred that the orthopox virus encodes a heterologous polypeptide antigen encoding a CD4+ and / or CD8+ T cell epitope against which the vaccinee has a pre-existing specific cellular immune response that was generated by a means other than by immunization with the said recombinant orthopox virus

The invention is now described by way of illustration only in the following examples in which the antigen is HBsAg. An Elisa assay was used to measure the anti-HBsAg antibody response. IFN-γ Elispot assays were used to measure T cell responses to whole antigen (HBsAg) or peptide (CD8+ epitope IPQSLDSWWTSL). Statistical analysis was provided using the SPSS 11.0 program to perform Mann-Witney U tests (non-parametric test, 2 independent samples). Exact significance [2* 1-tailed].

A number of commercially available vaccines are available for Hepatitis B that induce protective levels of antibodies. However, not all patients successfully sero-convert to protective levels (Alper FE *et al* 1995 exp clin immunogenet 12:171-81). These vaccines induce high levels of anti-HBsAg antibody but a minimal specific effector T-cell response as measured, for example, by the widely used interferon-gamma ELISPOT assay. As discussed above, one of the current challenges facing vaccinology is the identification of means to induce strong humoral and cellular response concurrently. Both responses may be of value in preventing and treating a number of diseases including hepatitis B virus infection. Inducing both responses may provide protection to the percentage of the population who fail to sero-convert to the standard alum-adjuvanted commercially available vaccine (for example, the Engerix B product), and in the treatment of persistent hepatitis B virus infection.

DNA and MVA has been used for a number of years in laboratory animals and in many human clinical trials as a vector for the antigen or antigens of interest (Schneider *et al* 1999 imm.Rev, Hanke, Vaccine. 2002 May 6: 20(15): 199-8,. Matteo 1999 J immunol 163:4058) in order to induce strong effector CD4+ and CD8+ T cell responses.

Experiments

Experiment 1

Aim:

Induction of a strong humoral response to HBsAg while still maintaining a strong cellular response by combining a vaccine to induce protective antibody levels (Engerix-B contains HBsAg and PreS regions of HBV adsorbed to Alum) with a T-cell inducing regime (DNA prime and MVA boost, both containing HBsAg and PreS regions of HBV).

1.1 Antibody Responses

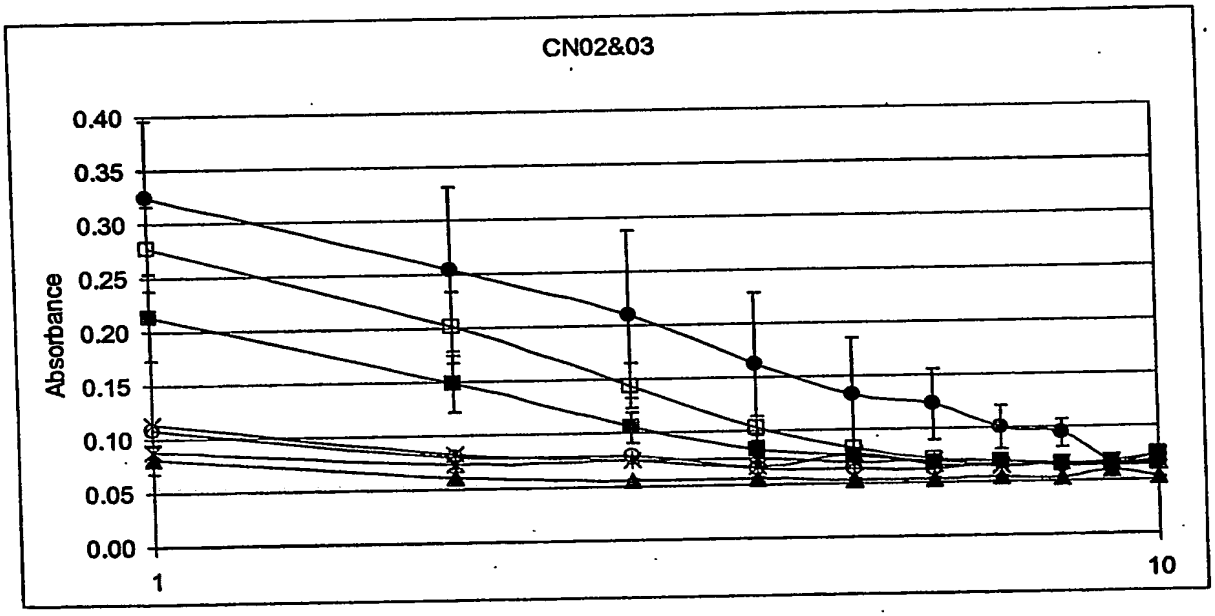

Figure 1

n=3-6 +/- SEM

PRIME
1. ▲ DNA.HBs i.m.
2. ○ Nil
3. ● DNA.HBs i.m.
4. □ DNA.HBs i.m.
5. ■ DNA.HBs i.m.
6. × DNA.HBs i.m.
7. * Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

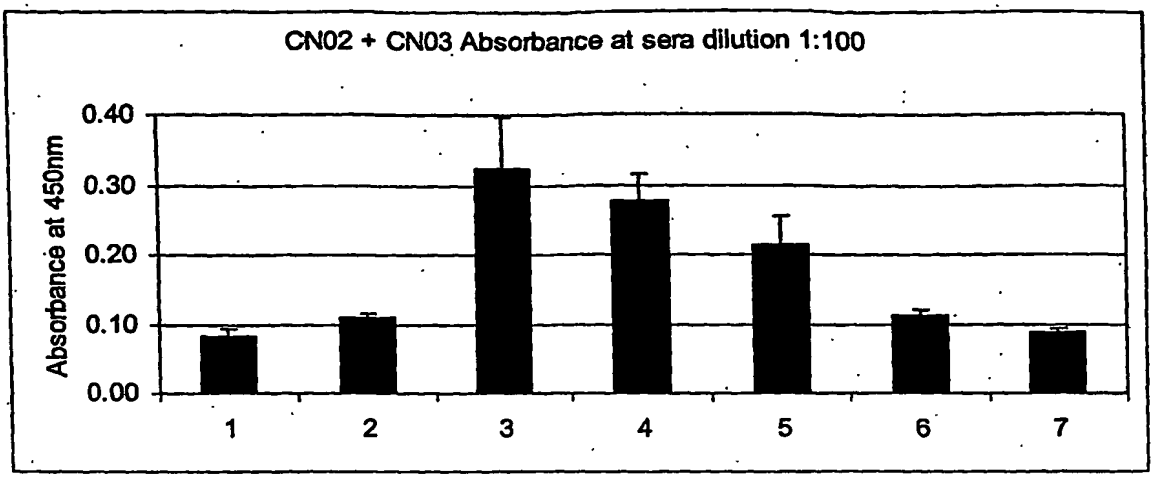

Figure 2

n=3-6 +/- SEM

PRIME	BOOST
1. DNA.HBs i.m.	MVA.HBs i.v.
2. Nil	Engerix-B s.c.
3. DNA.HBs i.m.	MVA.HBs + Engerix-B s.c.
4. DNA.HBs i.m.	MVA.HBs i.v. Engerix-B s.c.
5. DNA.HBs i.m.	Engerix-B s.c.
6. DNA.HBs i.m.	MVA.HBs + Alum s.c.
7. Naïve	

MVA.HBs Adjuvants Engerix-B

DNA.HBs prime followed by MVA.HBs mixed with Engerix B and administered s.c. produced the strongest antibody response. The next best immunisation regime was DNA.HBs prime followed by MVA.HBs i.v. and Engerix B s.c.

This indicated that, surprisingly, MVA.HBs when mixed with Engerix-B (group 3) was further adjuvanting the HBsAg in the Engerix-B vaccine leading to a stronger antibody response than was observed when the Engerix-B and MVA.HBs were administered separately (group 4). This is further supported by DNA.HBs priming followed by Engerix-B boosting (group 5) which gave lower responses than when MVA.HBs was included in the boost (group 3).

DNA.HBs priming increases antibody responses to Engerix-B

DNA.HBs however, was in part responsible for the increase in antibody responses. One shot of Engerix B at week 2 (no immunization at prime) gave significantly lower antibody responses than DNA.HBs priming followed by boosting with Engerix-B with or without MVA.HBs (s.c. or i.v) ($p=0.026$, 0.002, 0.002 respectively).
DNA.HBs priming followed by MVA.HBs boosting gave the lowest antibody response, not significantly different from unimmunised animals ($p=0.931$).

MVA.HBs boosts antibody response when administered at the same time as Engerix B either at the same or different sites.

DNA.HBs priming increases the antibody response to a single shot of Engerix B given 2 weeks following priming.

1.2 T-Cell Responses

1.2.1 Peptide stimulated splenocytes

DNA.HBs prime and MVA.HBs boost gave the strongest T cell responses but not significantly different to DNA.HBs priming followed by boosting with Engerix-B and MVA.HBs(s.c. or i.v) (p=0.343 both s.c. and i.v.).

DNA.HBs priming followed by boosting with Engerix-B and MVA.HBs i.v was significantly better than DNA.HBs priming followed by Engerix-B boosting (p=0.029). This suggests that MVA.HBs is responsible for inducing high levels of specific T-cells and that this response is not significantly reduced when combined with Engerix-B.

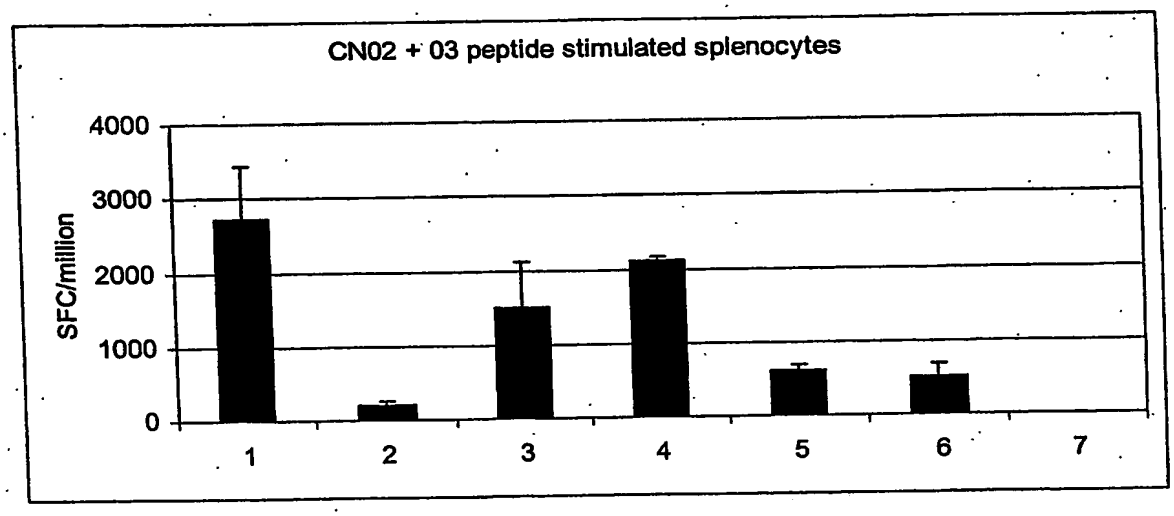

Figure 3

n=3-6 +/- SEM

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

1.2.2 HBsAg Stimulated Splenocytes

A similar patter to peptide stimulated splenocytes is seen upon HBsAg stimulation, although much lower overall response levels, probably because processing and presentation of the entire protein is incomplete; no processing is required for peptide presentation.

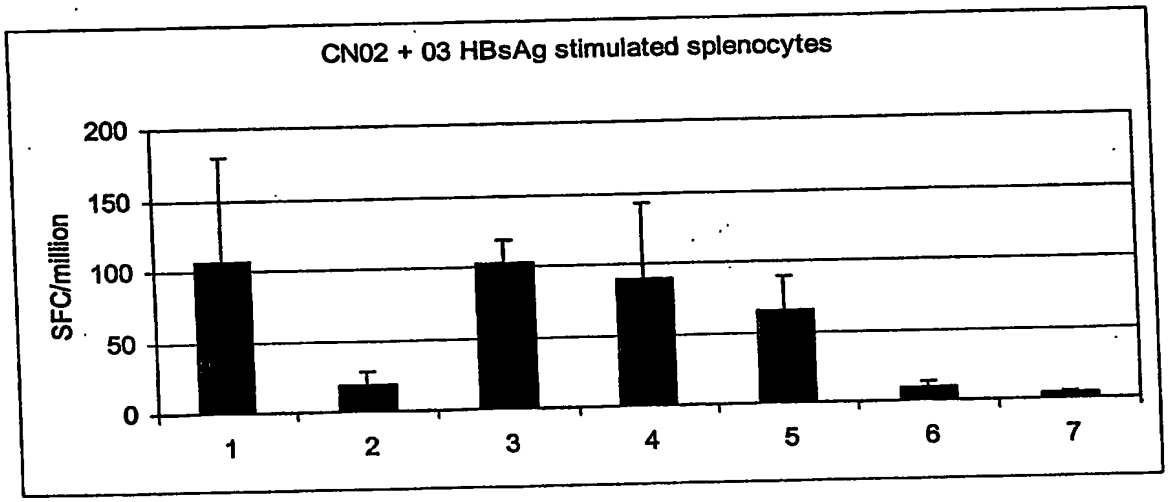

Figure 4

n=3-6 +/- SEM

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

1.2.3 Peptide Stimulated Lymph Nodes

Again, a similar pattern to peptide stimulated splenocytes. Cells from cervical and axial lymph nodes for each group were pooled. The strongest response was seen in DNA.HBS primed and MVA.HBs i.v. and Engerix-B boosted animals.

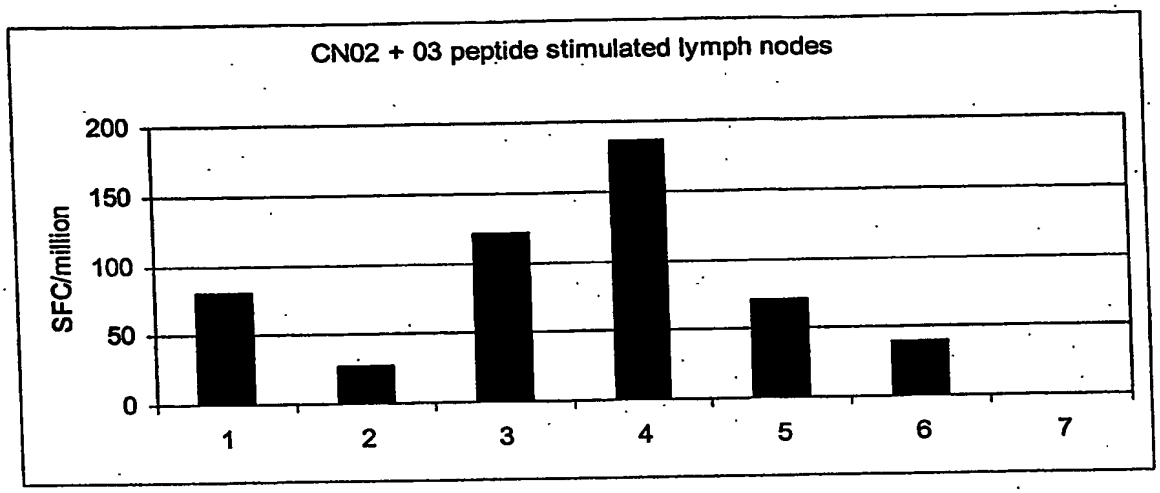

Figure 5

n= cells from 3-6 animals pooled

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

1.2.4 HBsAg Stimulated Lymph Nodes

Responses to whole antigen was well below 50 SFC/million except for animals primed with DNA.HBS and boosted s.c. with MVA.HBs and Engerix-B where cell numbers were 212 SFC/million.

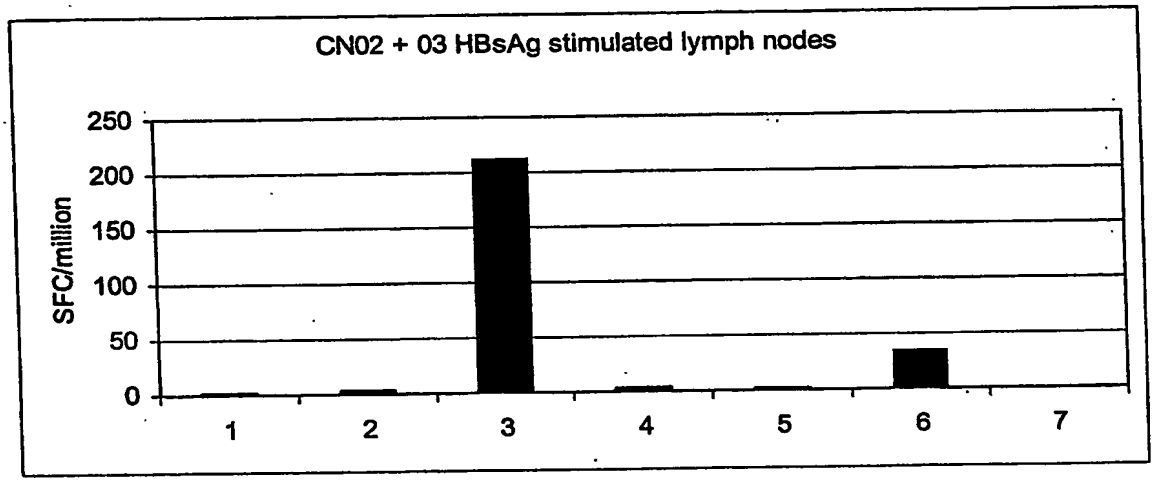

Figure 6

n= cells from 3-6 animals pooled

PRIME
1. DNA.HBs i.m.
2. Nil
3. DNA.HBs i.m.
4. DNA.HBs i.m.
5. DNA.HBs i.m.
6. DNA.HBs i.m.
7. Naïve

BOOST
MVA.HBs i.v.
Engerix-B s.c.
MVA.HBs + Engerix-B s.c.
MVA.HBs i.v. Engerix-B s.c.
Engerix-B s.c.
MVA.HBs + Alum s.c.

Experiment 2

To further increase Ab levels by including Engerix-B in the prime and still maintain T-cell responses. To establish whether the enhancement still apply when the route of MVA administration is changed to intradermal (id) applies and to bring it closer to the subcutaneous (sc) immunisation site of Engerix-B, so that both immunizations share the same draining lymph node. To establish whether an MVA vector not encoding an antigen (MVA.LacZ) boosts responses to HBsAg

2.1 Antibody responses

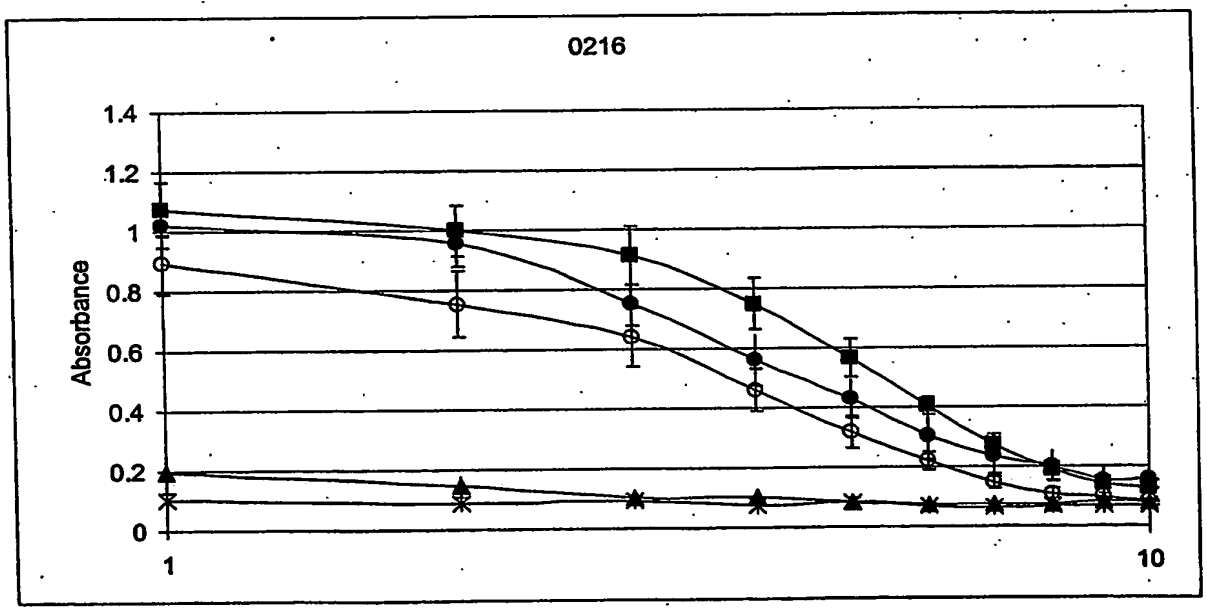

Figure 7

n=5 +/- SEM

PRIME
▲ DNA.HBs
○ DNA.HBs i.m. Engerix s.c.
● DNA.HBs i.m. Engerix s.c.
□ DNA.HBs i.m. Engerix s.c.
* Naive

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

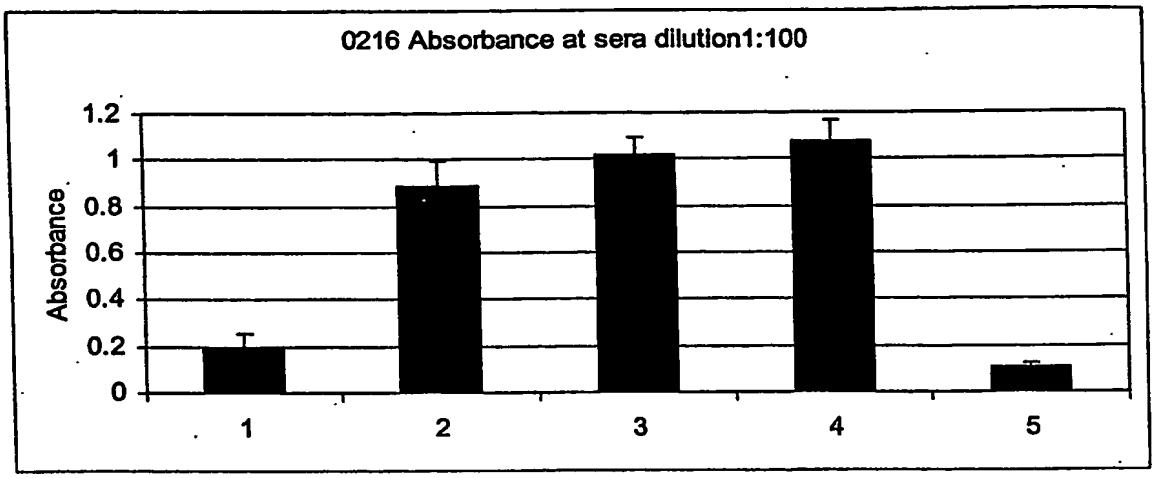

Figure 8

n=5 +/- SEM

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.
5. Naive

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

MVA .LacZ and MVA.HBs boosts antibody responses equally

DNA.HBs and Engerix-B priming combined with a boost of Engerix-B and either MVA.HBs(i.d or s.c) or MVA.LacZ s.c. increased antibody responses 10-fold compared to any immunisation regime in Experiment one.

The antibody responses to these three regimes were not significantly different from each other and it can therefore be concluded that MVA boosts antibody responses against HBsAg by acting as an adjuvant.

2.2 T-cell responses

2.2.1 Peptide Stimulated Splenocytes

DNA.HBs prime followed by MVA.HBs boost along with DNA.HBs and Engerix-B priming combined with a boost of Engerix-B s.c. and MVA.HBs i.d. give equally strong T-cell responses to peptide. However if MVA.HBs or MVA.LacZ is given s.c. with Engerix-B following DNA.HBs and Engerix-B priming this cellular response is abrogated. Therefore in order for strong cellular responses to be maintained the MVA needs to express the antigen present in the protein immunisation, in this case HBsAg. Priming with Engerix-B and DNA.HBs then boosting with MVA.HBs i.d. and Engerix-B s.c. significantly increases T-cell responses to peptide when compared with Exp 1 regime of DNA.HBs priming followed by boosting with MVA.HBs i.v. and Engerix-boost (p=0.004).

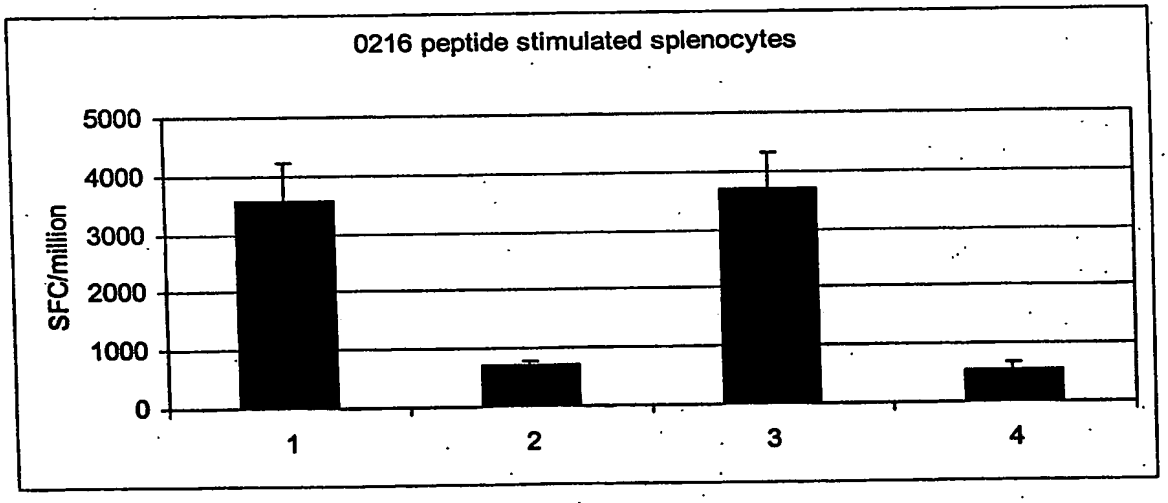

Figure 9

n=5 +/- SEM

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

2.2.2 HBsAg Stimulated Splenocytes

A similar pattern to that of peptide-stimulated splenocytes is seen upon HBsAg stimulation of splenocytes. The numbers are a lot lower than peptide stimulation. However, they are greater than those seen in experiment one.

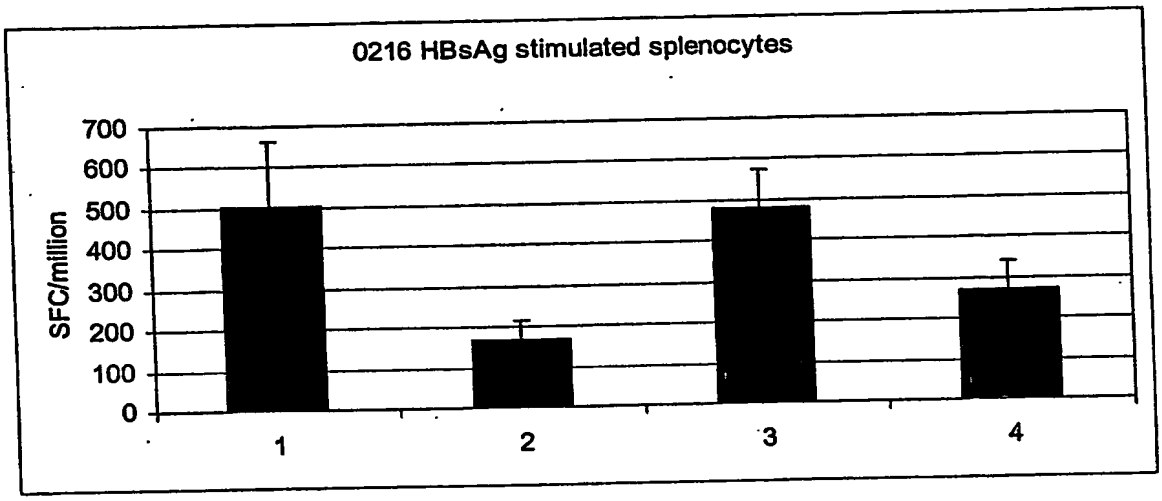

Figure 10

n=5 +/- SEM

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

2.2.3 Peptide Stimulated Lymph Nodes

As with Exp 1 when MVA.HBs is given at an alternate site to the Engerix boost the t-cell response is elevated in comparison to other immunisation regimes.

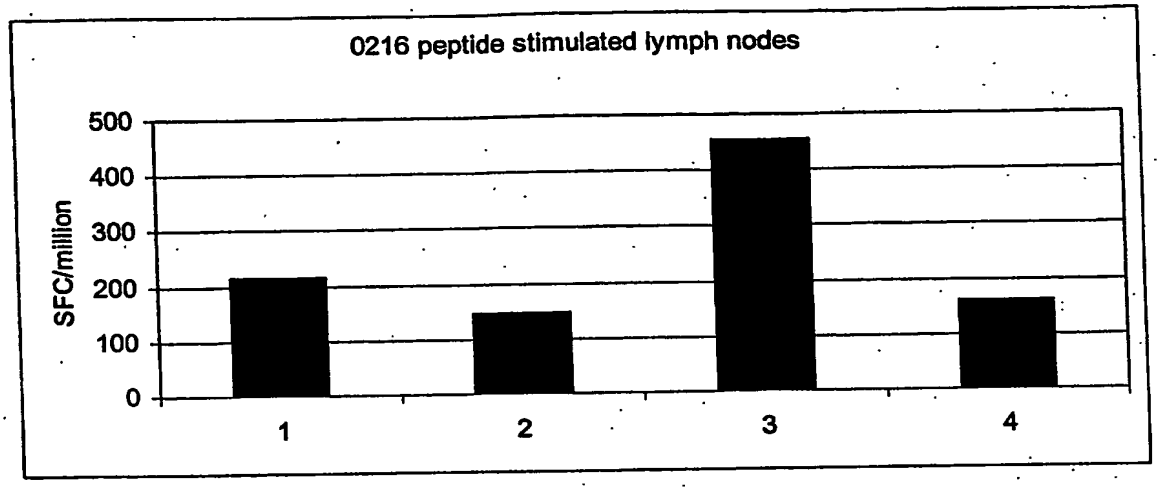

Figure 11

n= cells from 5 animals pooled

PRIME
1.DNA.HBs
2.DNA.HBs i.m. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.

BOOST
MVA.HBs i.d.
MVA.HBs s.c. Engerix s.c.
MVA.HBs i.d. Engerix s.c.
MVA.lacZ s.c. Engerix s.c.

2.2.4 HBsAg Stimulated Lymph Nodes

Same pattern as peptide stimulated LN's, but half the numbers. Again a much higher response(11 fold) when MVA.HBs is given i.d. compared to s.c. with Engerix-B. Although, in Exp 1 DNA.HBs prime followed by MVA.HBS and Engerix-B boost s.c. is the only regime that gives good responses to HBsAg stimulated LN's.

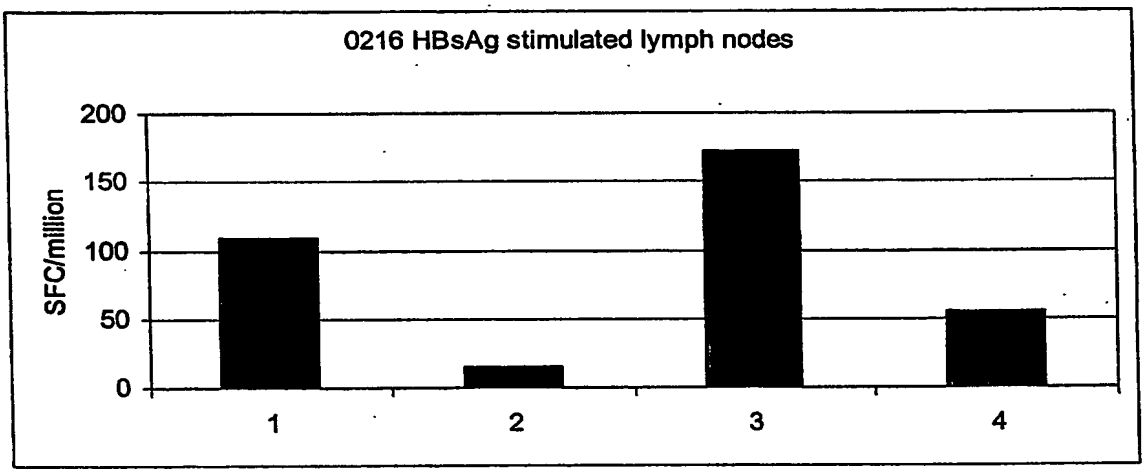

Figure 12

n= cells from 5 animals pooled

PRIME	BOOST
1.DNA.HBs	MVA.HBs i.d.
2.DNA.HBs i.m. Engerix s.c.	MVA.HBs s.c. Engerix s.c.
3. DNA.HBs i.m. Engerix s.c.	MVA.HBs i.d. Engerix s.c.
4. DNA.HBs i.m. Engerix s.c.	MVA.lacZ s.c. Engerix s.c.

Experiment 3

Aim:-

To establish whether a good cellular and Ab responses can be achieved in the absence of Alum using a homologous boost.

The same immunising agent and route was used for prime and boost.

3.1 Antibody responses

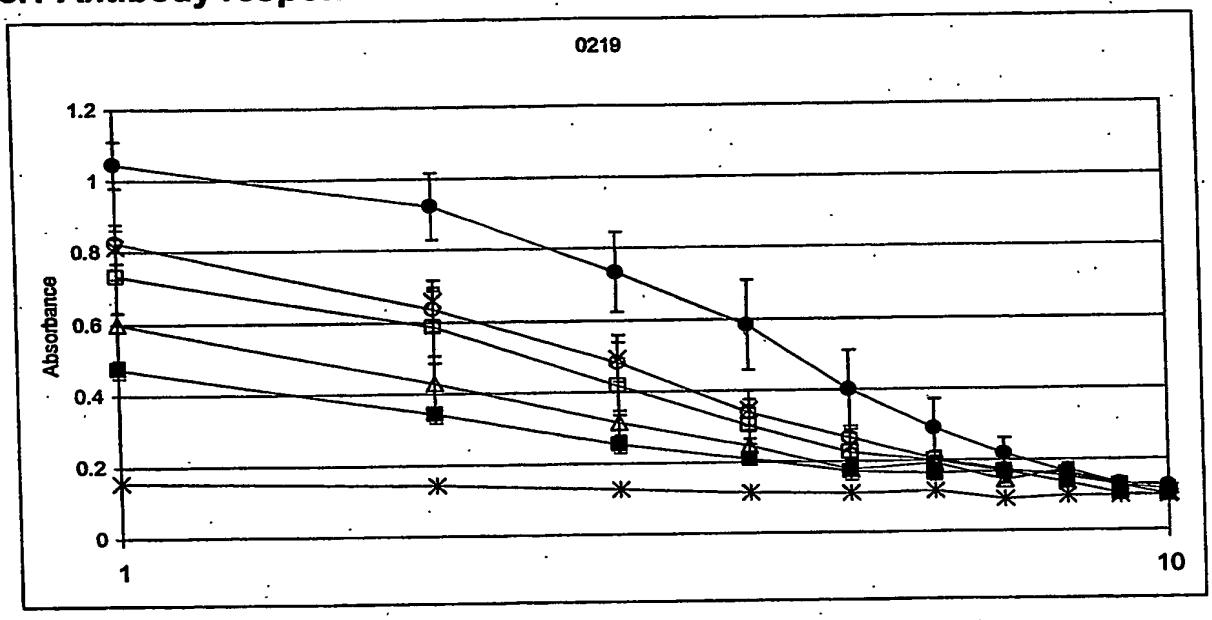

Figure 13

n=4 +/- SEM

PRIME
- ▲ HbsAg s.c.
- o HbsAg i.d.
- ● HbsAg + MVA.LacZ mixed i.d.
- □ Engerix-B s.c.
- ■ Eng-B s.c. MVA.LacZ i.d.
- × Eng-B + MVA.LacZ mix s.c.
- * Naïve

BOOST
- HbsAg s.c.
- HbsAg i.d.
- HbsAg + MVA.LacZ mixed i.d.
- Engerix-B s.c.
- Eng-B s.c. MVA.LacZ i.d.
- Eng-B + MVA.LacZ mix s.c.

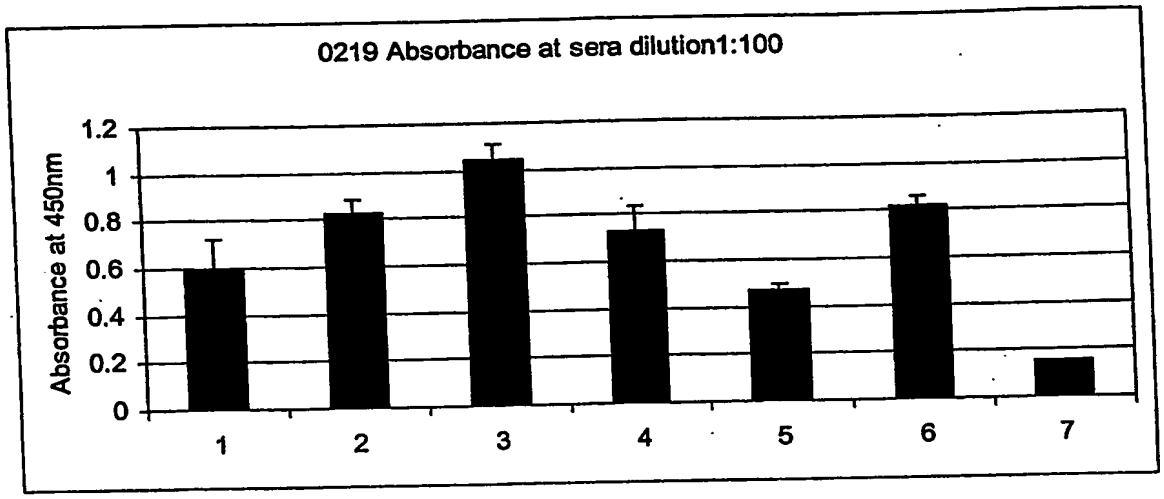

Figure 14

n=4 +/- SEM

PRIME
1.HbsAg s.c.
2.HbsAg I.d.
3.HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.
7.Naïve

BOOST
HbsAg s.c.
HbsAg I.d.
HbsAg + MVA.LacZ mixed i.d.
Engerix-B s.c.
Eng-B s.c. MVA.LacZ i.d.
Eng-B + MVA.LacZ mix s.c.

Highest level of antibodies were achieved with HBsAg mixed with MVA.LacZ and administered i.d. Levels were not significantly different to Exp 2 immunisations where DNA.HBs priming was followed by boosting with Engerix-B and MVA.HBs (i.d. or s.c.) or MVA.LacZ s.c. ($p= 0.905$, 0.19 and 0.905 respectively).

Two immunisations with Engerix-B produced lower antibody responses than HBsAg mixed with MVA.LacZ administered i.d ($p= 0.057$).

Homologous immunisation with HBsAg i.d. alone was not significantly different from Engerix-B alone or HBsAg and MVA.LacZ given i.d., ($p= 0.686$ and 0.114 respectively).

Engerix-B given with MVA.LacZ s.c (prime and boost) was significantly lower in two groups from Exp 1 that received DNA.HBs and Engerix-B priming combined with a boost of Engerix-B and either MVA.HBs i.d.. or MVA.LacZ s.c ($p= 0.032$ both). But not if the MVA.HBs was given s.c..($p=0.556$)

This indicates that DNA.HBs priming increases the antibody response to HBsAg .

High levels of antibody can be induced by priming with DNA.HBs and Engerix-B then boosting with MVA.LacZ or MVA.HBs and Engerix-B. These levels are comparable to those induced by two homologous immunisations of HBsAg and MVA.LacZ i.d.

3.2 T-cell Responses

3.2.1 Peptide Stimulated Splenocytes

T-cell responses to all vaccine regimes were very low(all well below 500 SFC/million) when compared to the best regimes of other Experiments.

Surprisingly, homologous prime/boost of Engerix-B mixed with MVA.LacZ and administered s.c yielded similar numbers to the **Spec2 ???** regime of DNA.HBs priming followed by boosting with Engerix-B and MVA.LacZ s.c. (average of 336 +/-113 SEM and 511 +/-173 SEM SFC/million respectively). This suggests that DNA.HBs combined with Engerix-B priming is not enough to increase cellular responses to HBsAg when the MVA combined with Engerix-B is non-specific, in this case MVA.LacZ given either s.c or i.d. The MVA administered at the time of boosting needs to contain HBs in order to attain high levels of specific T-cells.

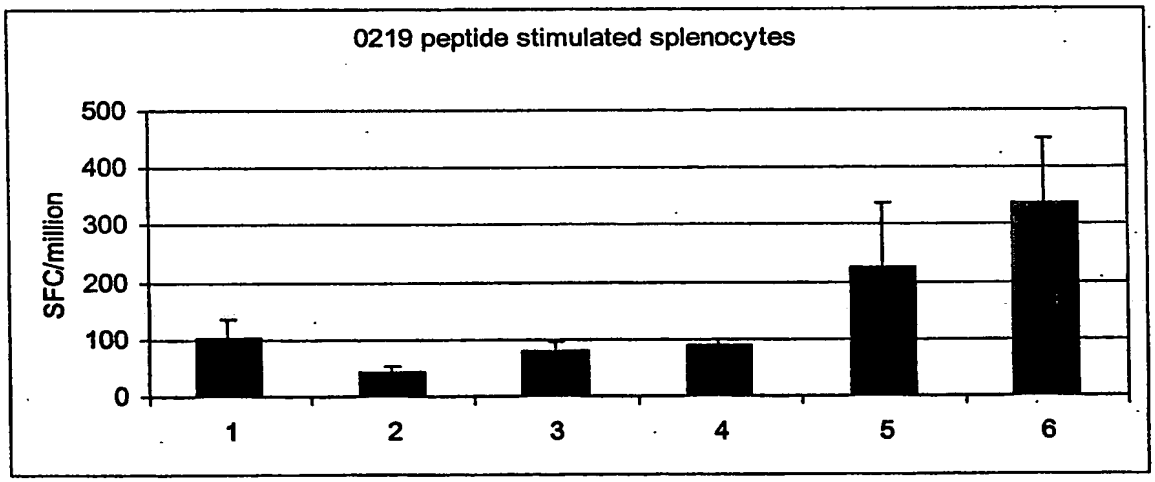

Figure 15
n=4 +/- SEM

PRIME	BOOST
1.HbsAg s.c.	HbsAg s.c.
2.HbsAg i.d.	HbsAg i.d.
3.HbsAg + MVA.LacZ mixed i.d.	HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.	Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.	Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.	Eng-B + MVA.LacZ mix s.c.

3.2.2 HBsAg Stimulated Splenoctyes

All below 150 SFC/million

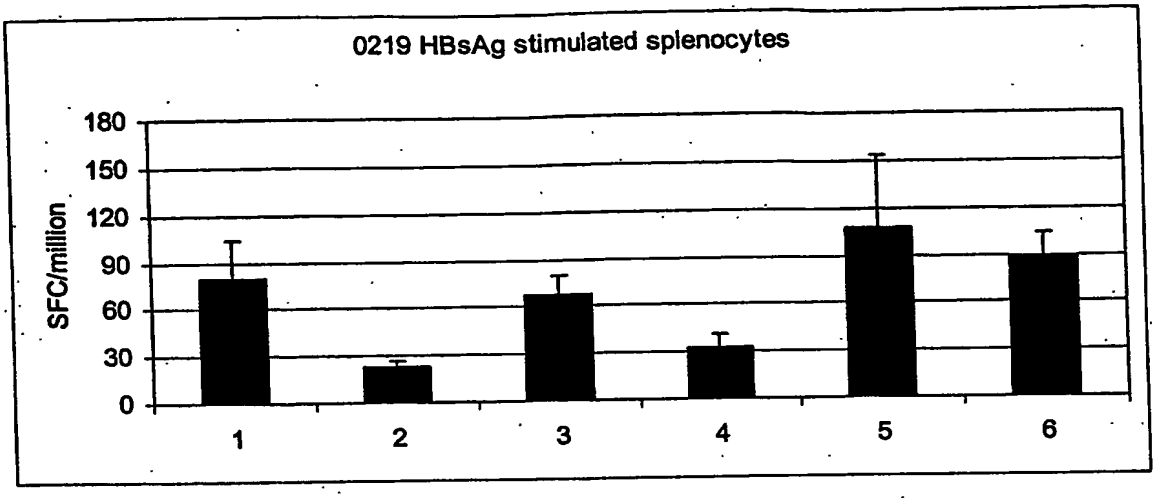

Figure 16

n=4 +/- SEM

PRIME
1.HbsAg s.c.
2.HbsAg I.d.
3.HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.

BOOST
HbsAg s.c.
HbsAg I.d.
HbsAg + MVA.LacZ mixed i.d.
Engerix-B s.c.
Eng-B s.c. MVA.LacZ i.d.
Eng-B + MVA.LacZ mix s.c.

3.2.3 Peptide Stimulated Lymph Nodes

Kept Axial and Cervical Lymph nodes separately. s.c. immunisations more likely to predominantly drain to the Axial LN's, i.d immunisations more likely to predominantly drain to cervical L.N's. HBsAg alone s.c gives higher responses in Axial L.N's. When given i.d. Cervical LN's have higher responses, equal to level in Axial LN's following s.c. immunisation.

Favourable regimes were either HBsAg mixed with MVA.LacZ and given i.d. or Engerix-B mixed with LacZ and given s.c.(363 and 343 respectively) in cervical LN's. Engerix-B given s.c. and MVA.LacZ given i.d. produced almost equal numbers of spots in cervical and axial LN's(147 and 132 respectively) and was similar in number to Engerix-B alone(83 cervical and 167 axial). This suggested that MVA.lacZ did not adjuvant Engerix-B in LN's when given i.d. but did in cervical LN's if mixed with Engerix-B and given s.c, therefore scenario the antigen of interest may need to be immunised at the same site as the MVA.LacZ to be adjuvanted by it.

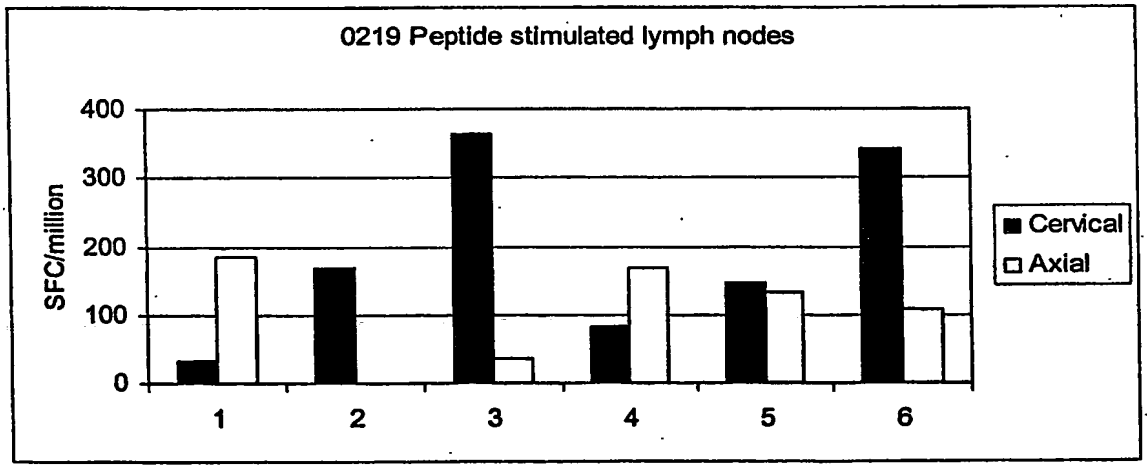

Figure 17
n= cells from 4 animals pooled

PRIME	BOOST
1.HbsAg s.c.	HbsAg s.c.
2.HbsAg i.d.	HbsAg i.d.
3.HbsAg + MVA.LacZ mixed i.d.	HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.	Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.	Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.	Eng-B + MVA.LacZ mix s.c.

3.2.4 HBsAg Stimulated Lymph Nodes

Similar pattern to peptide stimulation of LN's except even larger response in cervical LN's following i.d. immunisation of HBsAg mixed with MVA.LacZ(828 SFC/million).

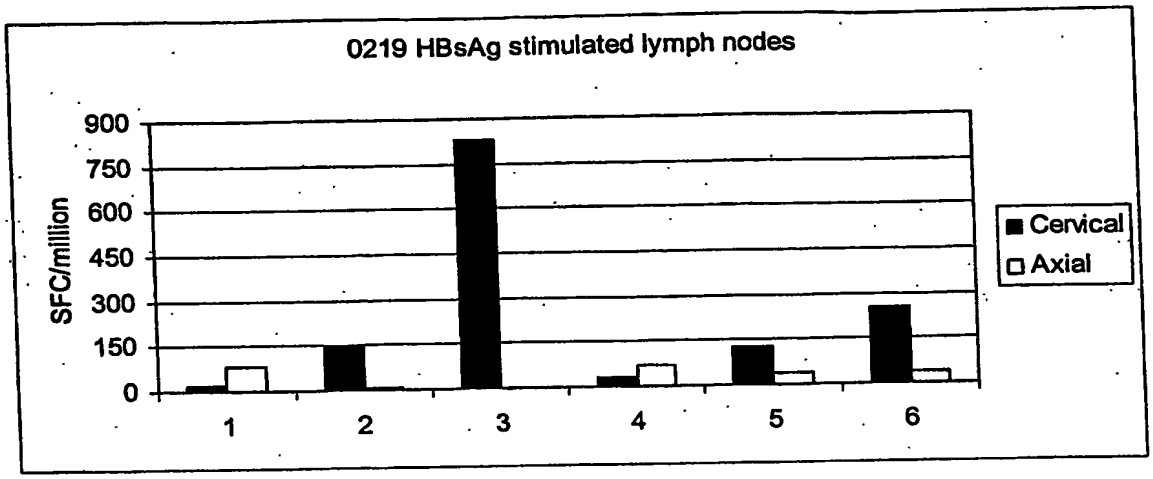

Figure 18

n= cells from 4 animals pooled

PRIME
1.HbsAg s.c.
2.HbsAg i.d.
3.HbsAg + MVA.LacZ mixed i.d.
4.Engerix-B s.c.
5.Eng-B s.c. MVA.LacZ i.d.
6.Eng-B + MVA.LacZ mix s.c.

BOOST
HbsAg s.c.
HbsAg i.d.
HbsAg + MVA.LacZ mixed i.d.
Engerix-B s.c.
Eng-B s.c. MVA.LacZ i.d.
Eng-B + MVA.LacZ mix s.c.

Conclusions From Experiments 1-3

DNA priming was shown to be important for inducing cellular and to a lesser extent antibody responses.
Exp 1 showed DNA.HBs priming followed by boosting with Engerix-B and MVA gave good cellular responses but low Ab levels.
Exp 2 showed that by including an Engerix-b prime with DNA then boosting with Engerix-B and MVA gave high T-cell and antibody levels

Exp 3 showed homologous prime/boost with MVA and HBsAg gave strong Ab responses but very low T-cell responses.

Antibody responses

a) MVA.LacZ and MVA.HBs can adjuvant the protein HBsAg to induce high levels of specific antibody against HBsAg.
b) DNA.HBs priming increases the antibody response to HBsAg when Engerix-B s.c. is concurrently used to prime followed by boosting with Engerix-B and MVA.lacZ s.c. (Experiment 3 group 4 vs Spec 3 group 6)
c) MVA.LacZ adjuvants Engerix-B to produce high antibody levels when both are mixed and immunised s.c. (Experiment 3, group 5 vs group 6)

T-Cell responses

a) DNA.HBs priming is required to induce high levels of specific T-cells. (Exp 3 vs Exp 1 & 2)
b) Following DNA priming highest T-cell responses are achieved by administering MVA.HBs i.v. or i.d, not s.c. (Spec 2)
c) Engerix-B used at prime with DNA HBs increases T-cell responses when boosted with MVA.HBs i.v. and Engerix-B s.c. (Spec 2 group 3 vs Spec 1 group 4)

A favourable strategy for inducing Antibody and T-cells to date:

Prime: DNA.HBs i.m. and Engerix-B s.c.
Boost: MVA.HBs i.d. and Engerix-B s.c.

T-cell responses: Not significantly different (P =0.61) from our 'gold standard' T-cell inducing regiment DNA.HBs i.m. prime and MVA.HBs i.v. or i.d. boost.

Antibody responses: Higher antibody levels than the 'gold standard' of homologous prime/boost with Engerix-B, of marginal statistical significance (p=0.06)

MVA may direct the immune system towards epitopes not normally targeted in current vaccine regimes thus overcoming the problem of non-responders.

Experiment 4

Aim: to retain HBsAg and MVA boost giving a good Ab responses, whilst improving the prime to restore cellular responses. Furthermore, an aim of this experiment was to establish whether the strong cellular response restored if MVA.HBs is used to adjuvant HBsAg at prime and boost, and whether FP.LacZ has an adjuvant activity for antibody induction.

4.1 Antibody Responses

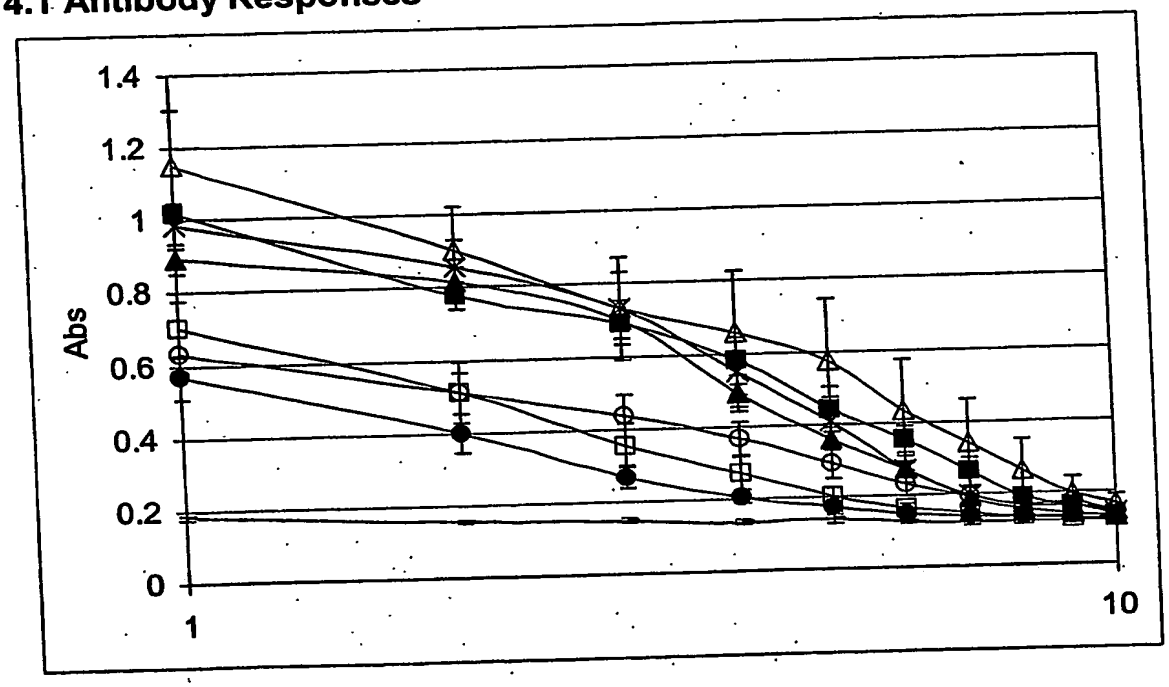

Figure 19

n=4 +/- SEM

PRIME
- Δ HBsAg + MVA.HBs mix i.d.
- ▲ DNA.HBs + HBsAg mix i.d.
- □ Engerix-B 5ug s.c.
- ■ DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
- ○ DNA.HBs i.m. & HBsAg i.d..
- ● DNA.HBs i.m. & Engerix-B s.c.
- * HBsAg + MVA.LacZ mix i.d.
- — Naive

BOOST
- HBsAg + MVA.HBs mix i.d
- HBsAg + MVA.HBs mix i.d.
- Engerix-B 5ug s.c.
- HBsAg + MVA.HBs mix i.d.
- HBsAg + MVA.HBs mix i.d.
- Engerix-B s.c. + MVA.HBs i.d.
- HBsAg + FP9.LacZ mix i.d.

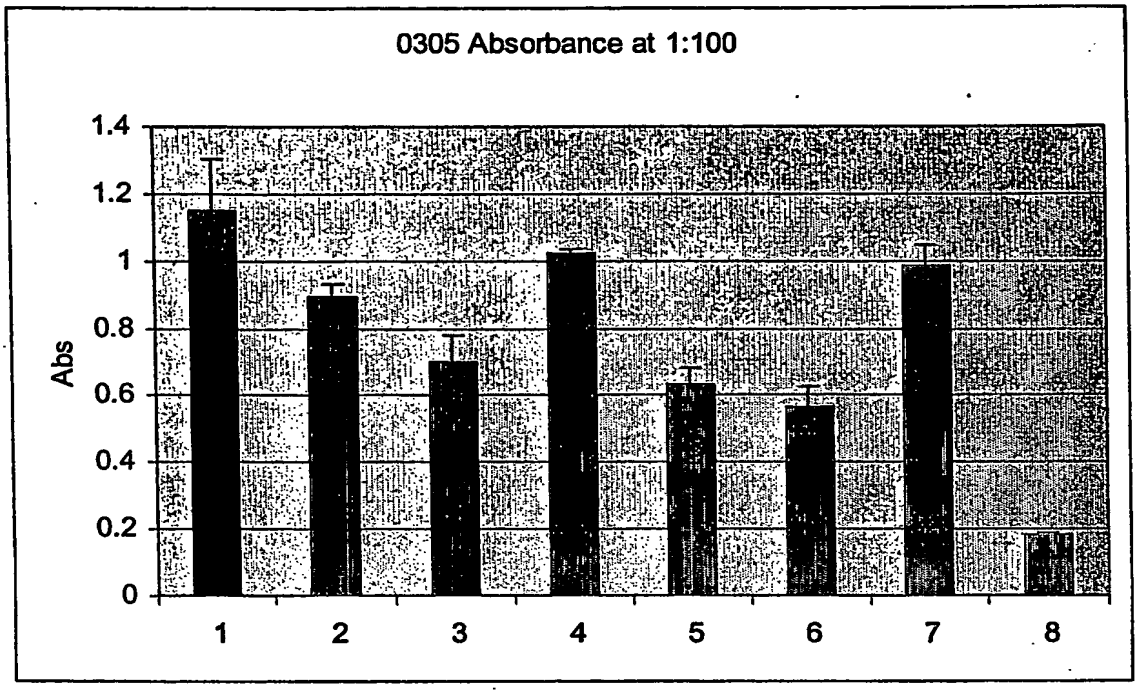

Figure 20

n=4 +/- SEM

	PRIME	BOOST
1	HBsAg + MVA.HBs mix i.d.	HBsAg + MVA.HBs mix i.d
2	DNA.HBs + HBsAg mix i.d.	HBsAg + MVA.HBs mix i.d.
3	Engerix-B 5ug s.c.	Engerix-B 5ug s.c.
4	DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d.
5	DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
6	DNA.HBs i.m. & Engerix-B s.c.	Engerix-B s.c. + MVA.HBs i.d.
7	HBsAg + MVA.LacZ mix i.d.	HBsAg + FP9.LacZ mix i.d.
8.	Naive	

4.2 T-cell Responses

4.2.1 Peptide Stimulated Splenocytes

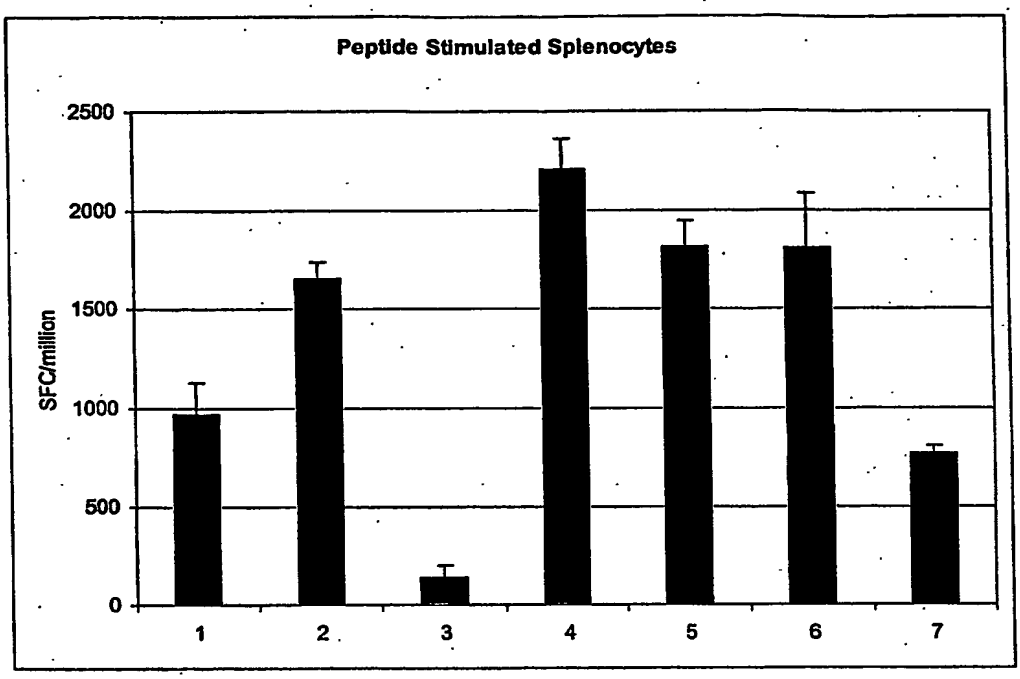

Figure 21

n=4 +/- SEM

PRIME	BOOST
1 HBsAg + MVA.HBs mix i.d.	HBsAg + MVA.HBs mix i.d
2 DNA.HBs + HBsAg mix i.d.	HBsAg + MVA.HBs mix i.d.
3 Engerix-B 5ug s.c.	Engerix-B 5ug s.c.
4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d.
5 DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
6 DNA.HBs i.m. & Engerix-B s.c.	Engerix-B s.c. + MVA.HBs i.d.
7 HBsAg + MVA.LacZ mix i.d.	HBsAg + FP9.LacZ mix i.d.

4.2.2 HBsAg stimulated splenocytes

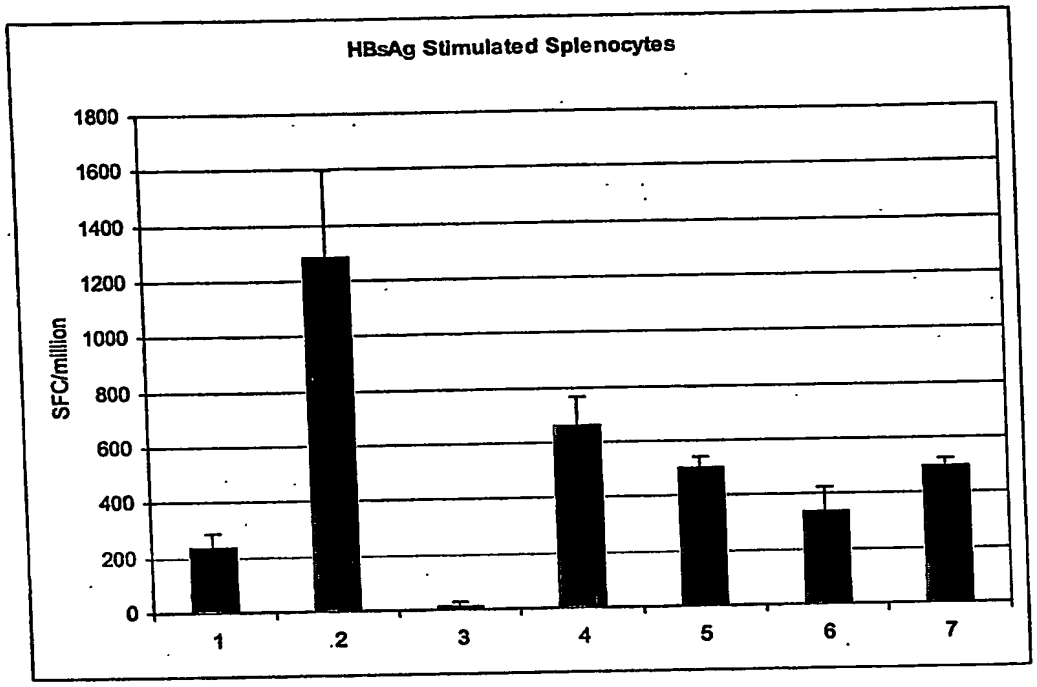

Figure 22

n=4 +/- SEM

PRIME
1 HBsAg + MVA.HBs mix i.d.
2 HBsAg + pSG2.HBs mix i.d.
3 Engerix-B 5ug s.c.
4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
5 DNA.HBs i.m. & HBsAg i.d.
6 DNA.HBs i.m. & Engerix-B s.c.
7 HBsAg + MVA.LacZ mix i.d.

BOOST
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
Engerix-B 5ug s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
Engerix-B s.c. + MVA.HBs i.d.
HBsAg + FP9.LacZ mix i.d.

4.2.3 Peptide Stimulated lymph nodes

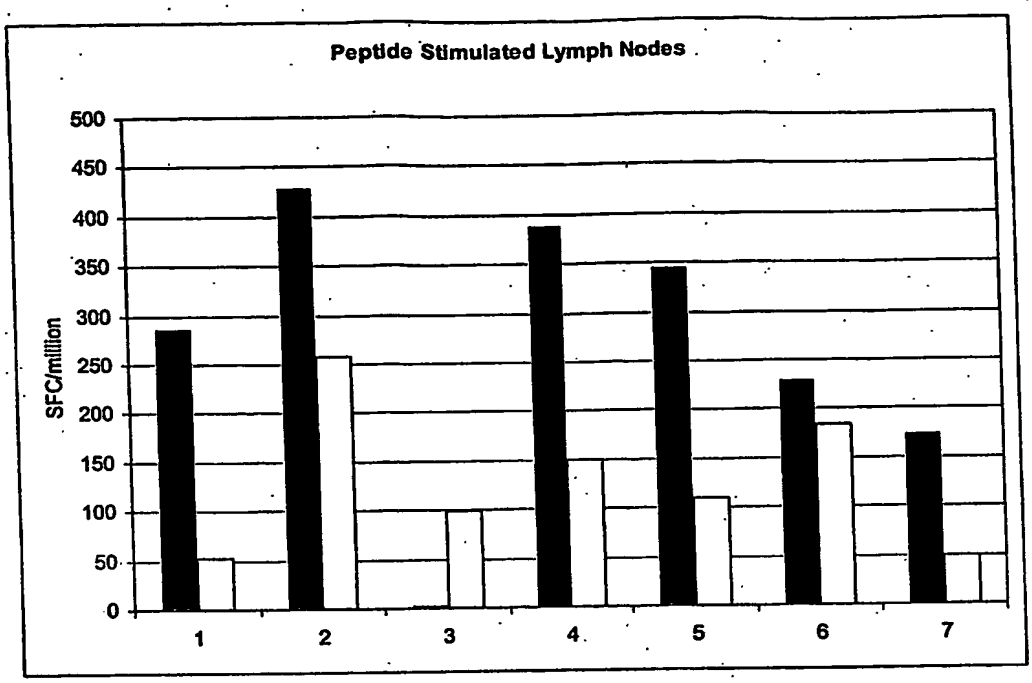

Figure 23

n= cells from 4 animals pooled

PRIME	BOOST
1 HBsAg + MVA.HBs mix i.d.	HBsAg + MVA.HBs mix i.d
2 DNA.HBs + HBsAg mix i.d.	HBsAg + MVA.HBs mix i.d.
3 Engerix-B 5ug s.c.	Engerix-B 5ug s.c.
4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d.
5 DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
6 DNA.HBs i.m. & Engerix-B s.c.	Engerix-B s.c. + MVA.HBs i.d.
7 HBsAg + MVA.LacZ mix i.d.	HBsAg + FP9.LacZ mix i.d.

4.2.4 HBsAg Stimulated lymph nodes

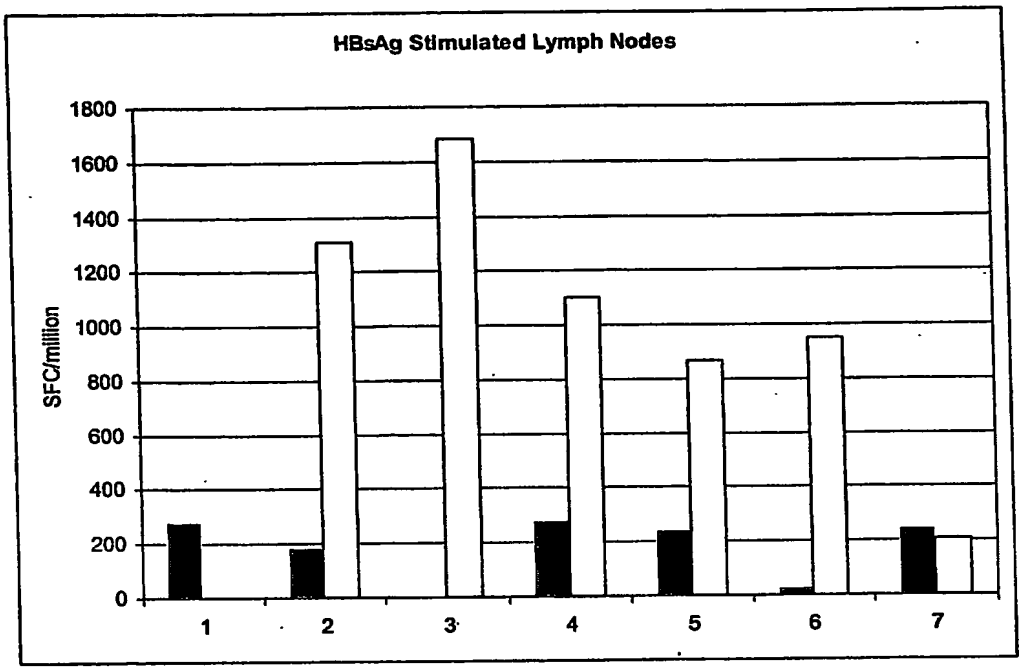

Figure 24

n= cells from 4 animals pooled

PRIME	BOOST
1 HBsAg + MVA.HBs mix i.d.	HBsAg + MVA.HBs mix i.d
2 DNA.HBs + HBsAg mix i.d.	HBsAg + MVA.HBs mix i.d.
3 Engerix-B 5ug s.c.	Engerix-B 5ug s.c.
4 DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d.
5 DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
6 DNA.HBs i.m. & Engerix-B s.c.	MVA.HBs i.d. + Engerix-B s.c.
7 HBsAg + MVA.LacZ mix i.d.	HBsAg + FP9.LacZ mix i.d.

Experiment 4 demonstrates that:

1. FP9 has adjuvant activity for antibody induction to the co-administered antigen – compared groups 4 and 5.
2. A heterologous prime-boost regime is required for optimal T cell induction; compare T cell responses in spleen in groups 1 and 2.

Summary of results from experiments 1-4

For ease of reference, the results of experiments 1-4 are compared, using the following key:

PRIME	BOOST
1. DNA.HBs i.m.	MVA.HBs i.v.
2. Nil	Engerix-B s.c.
3. DNA.HBs i.m.	MVA.HBs + Engerix-B s.c.
4. DNA.HBs i.m.	MVA.HBs i.v. Engerix-B s.c.
5. DNA.HBs i.m.	Engerix-B s.c.
6. DNA.HBs i.m.	MVA.HBs + Alum s.c.
7. DNA.HBs	MVA.HBs i.d.
8. DNA.HBs i.m. Engerix s.c.	MVA.HBs s.c. Engerix s.c.
9. DNA.HBs i.m. Engerix s.c.	MVA.HBs i.d. Engerix s.c.
10. DNA.HBs i.m. Engerix s.c.	MVA.lacZ s.c. Engerix s.c.
11. HbsAg s.c.	HbsAg s.c.
12. HbsAg I.d.	HbsAg I.d.
13. HbsAg + MVA.LacZ mixed i.d.	HbsAg + MVA.LacZ mixed i.d.
14. Engerix-B s.c.	Engerix-B s.c.
15. Eng-B s.c. MVA.LacZ i.d.	Eng-B s.c. MVA.LacZ i.d.
16. Eng-B + MVA.LacZ mix s.c.	Eng-B + MVA.LacZ mix s.c.
17. HBsAg + MVA.HBs mix i.d.	HBsAg + MVA.HBs mix i.d
18. HBsAg + pSG2.HBs mix i.d.	HBsAg + MVA.HBs mix i.d.
19. Engerix-B 5ug s.c.	Engerix-B 5ug s.c.
20. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d.
21. DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
22. DNA.HBs i.m. & Engerix-B s.c.	MVA.HBs i.d. + Engerix-B s.c.
23. HBsAg + MVA.LacZ mix i.d.	HBsAg + FP9.LacZ mix i.d.

Antibody responses

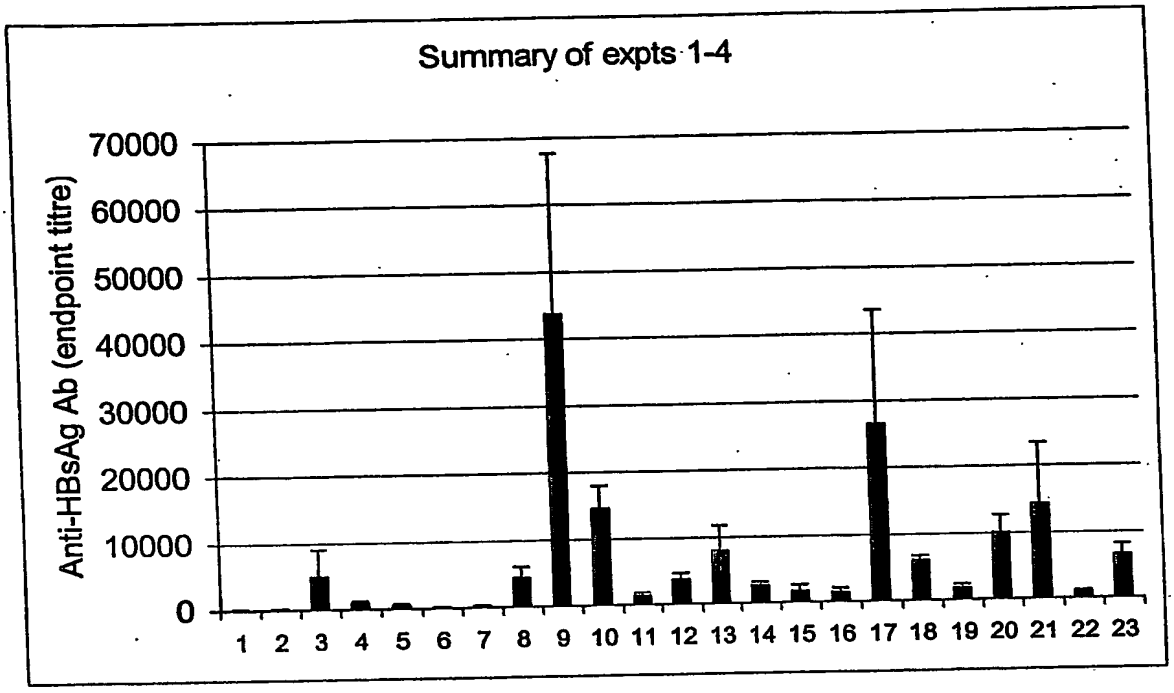

Figure 25

n=3-6 +/- SEM

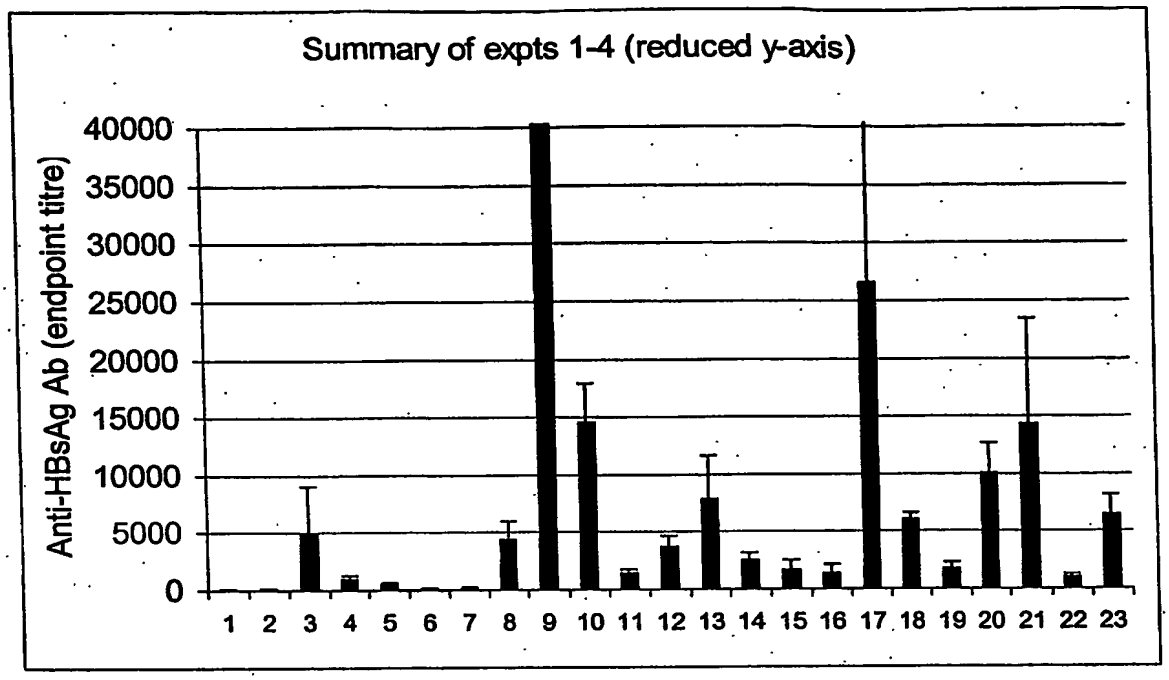

Figure 26

n=3-6 +/- SEM

Cellular responses

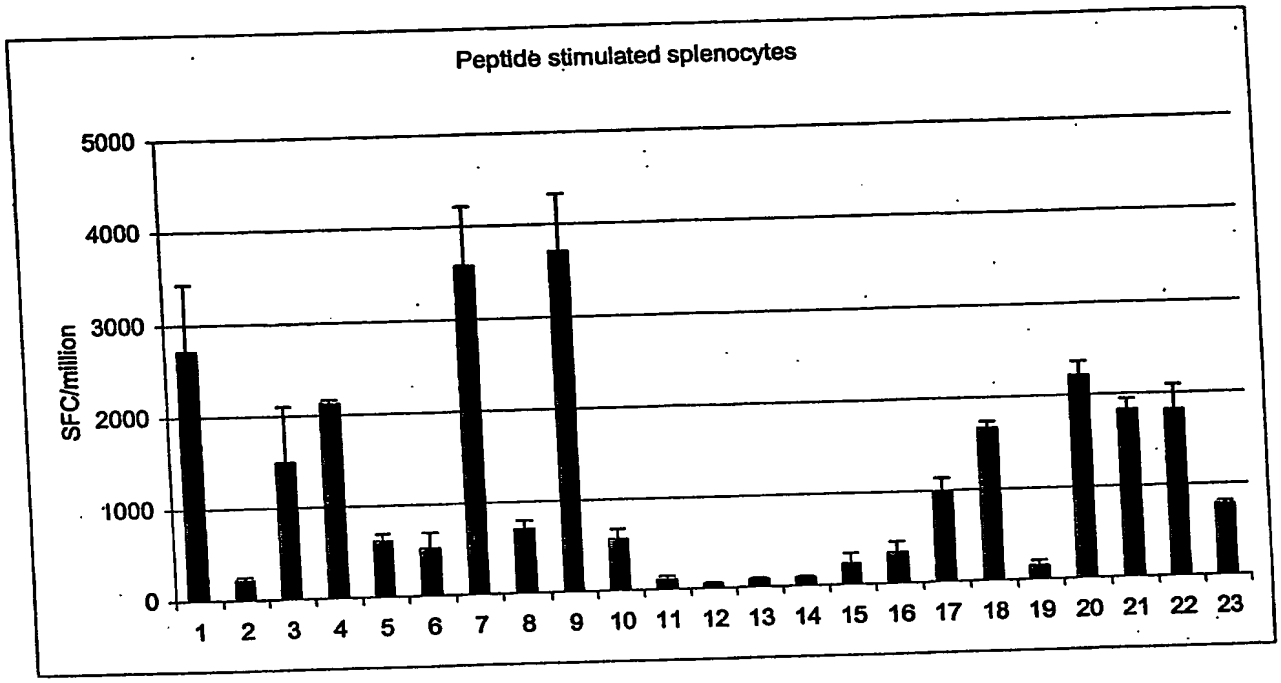

Figure 27

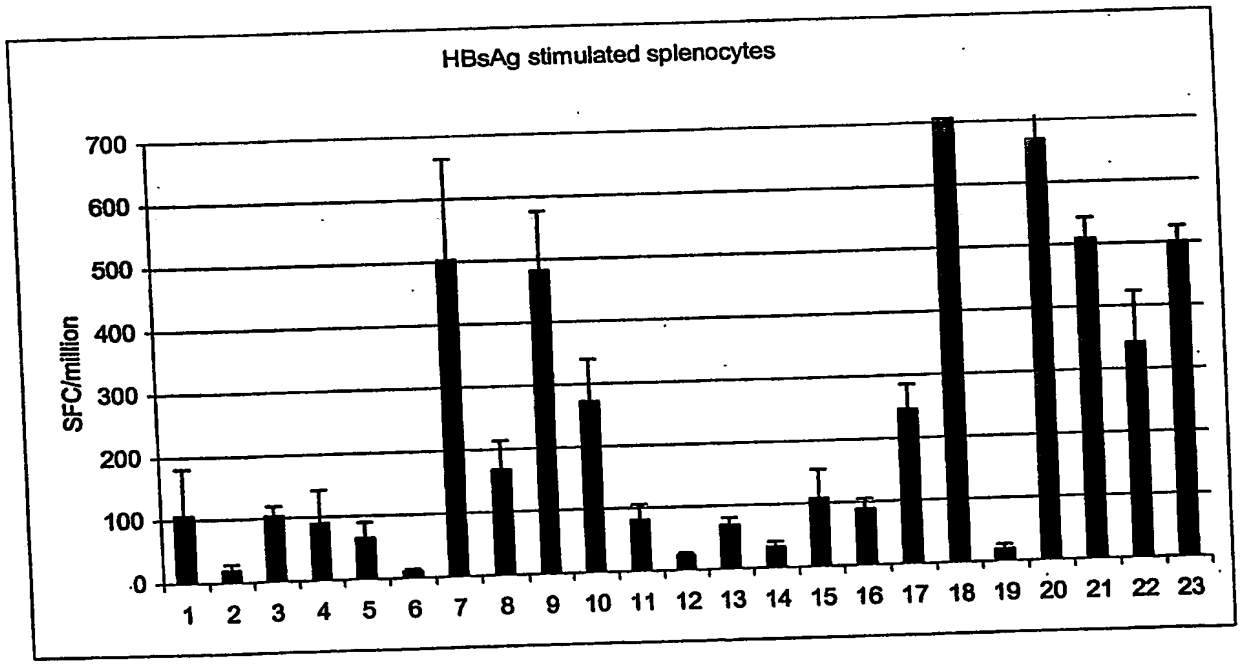

Figure 28

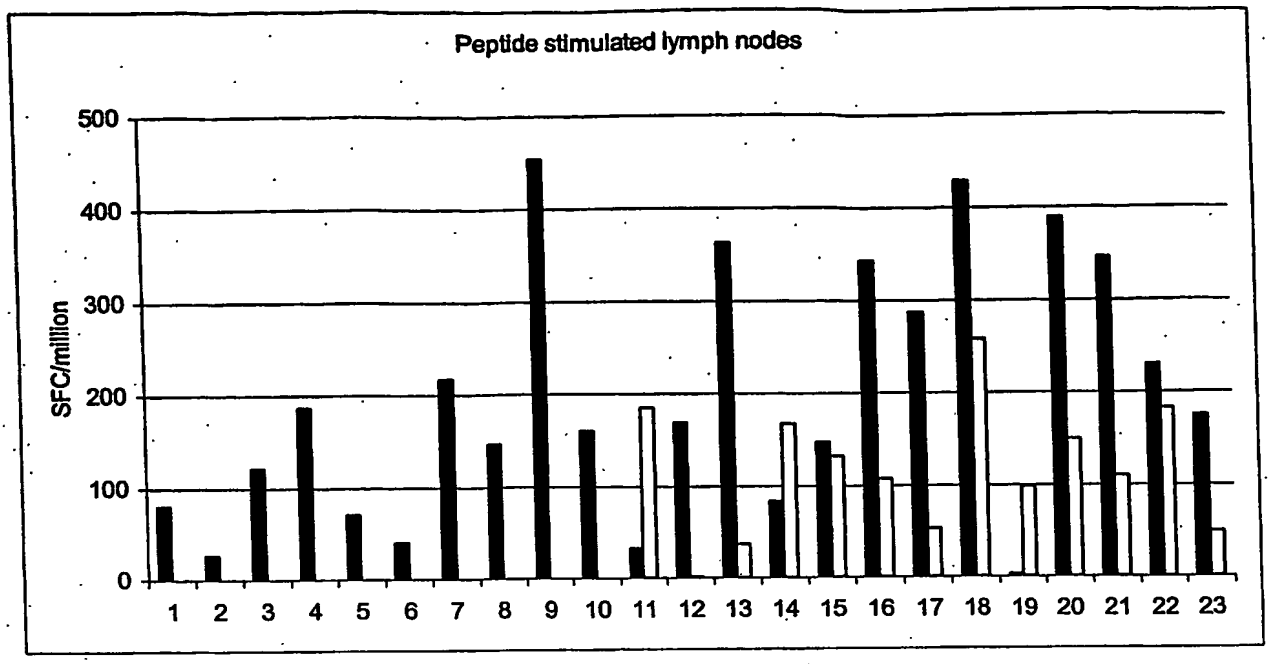

Figure 29

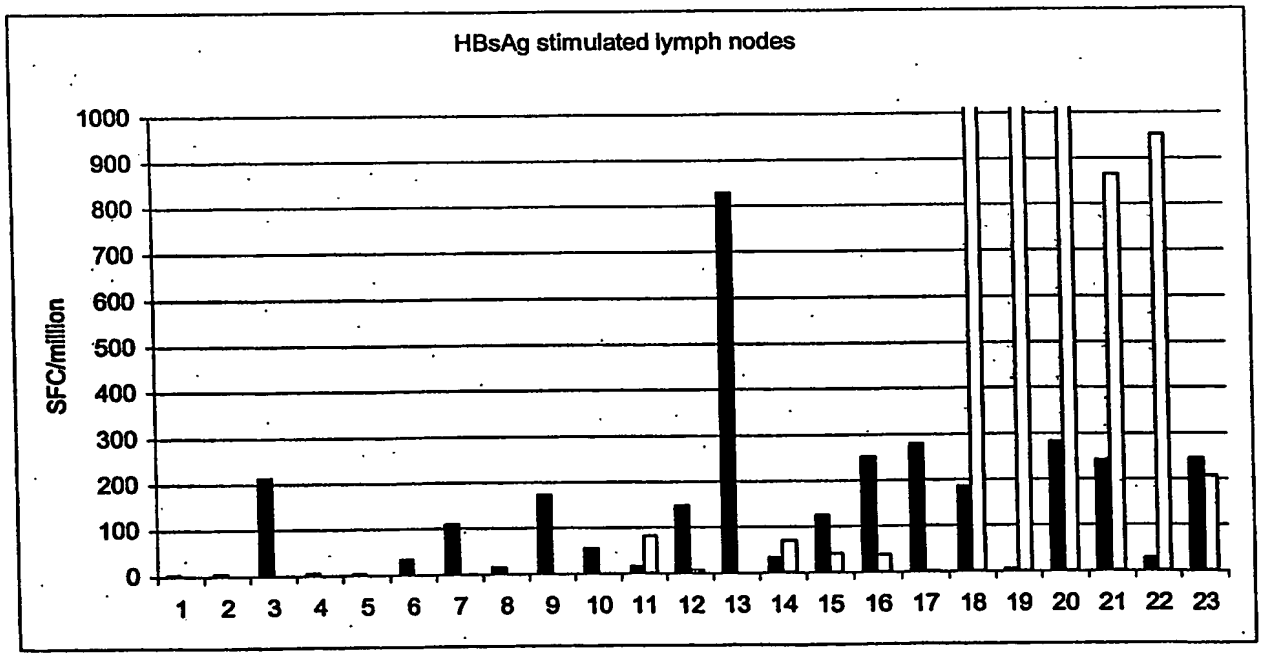

Figure 30

Experiment 5

Favourable antibody and cellular inducing regimes from experiments 1-4 were compared.

5.1 Antibody Responses

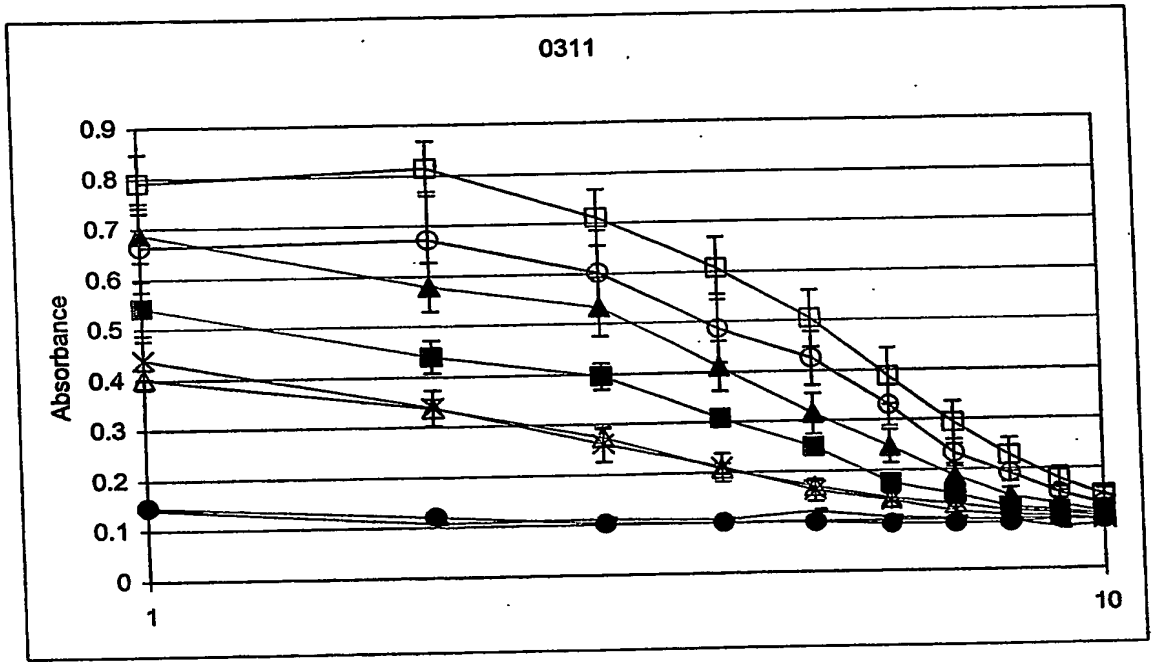

Figure 31

n=4 +/- SEM

PRIME

Δ DNA.HBs i.m. Engerix s.c.
▲ HBsAg + pSG2.HBs mix i.d.
□ DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
■ DNA.HBs i.m. & HBsAg i.d.
o HBsAg + MVA.HBs mix i.d
● DNA.HBs i.m.
* Engerix-B s.c.
— Naive

BOOST

MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

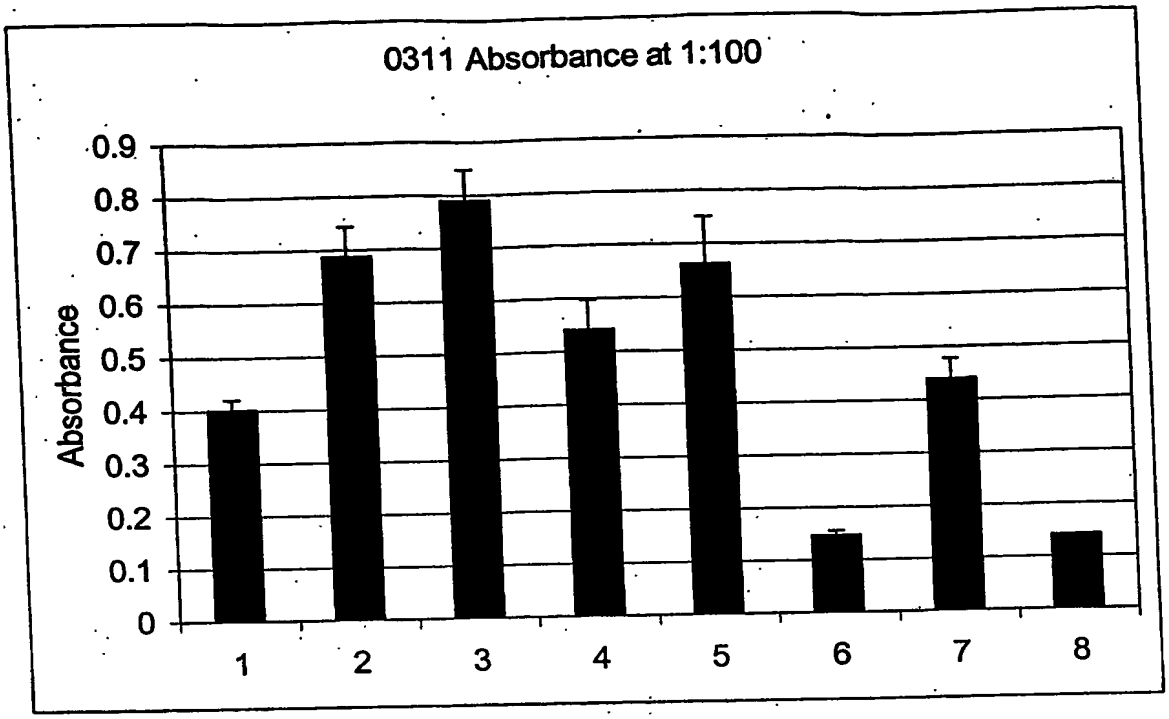

Figure 32

n=3-4 +/- SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.
8. Naive

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

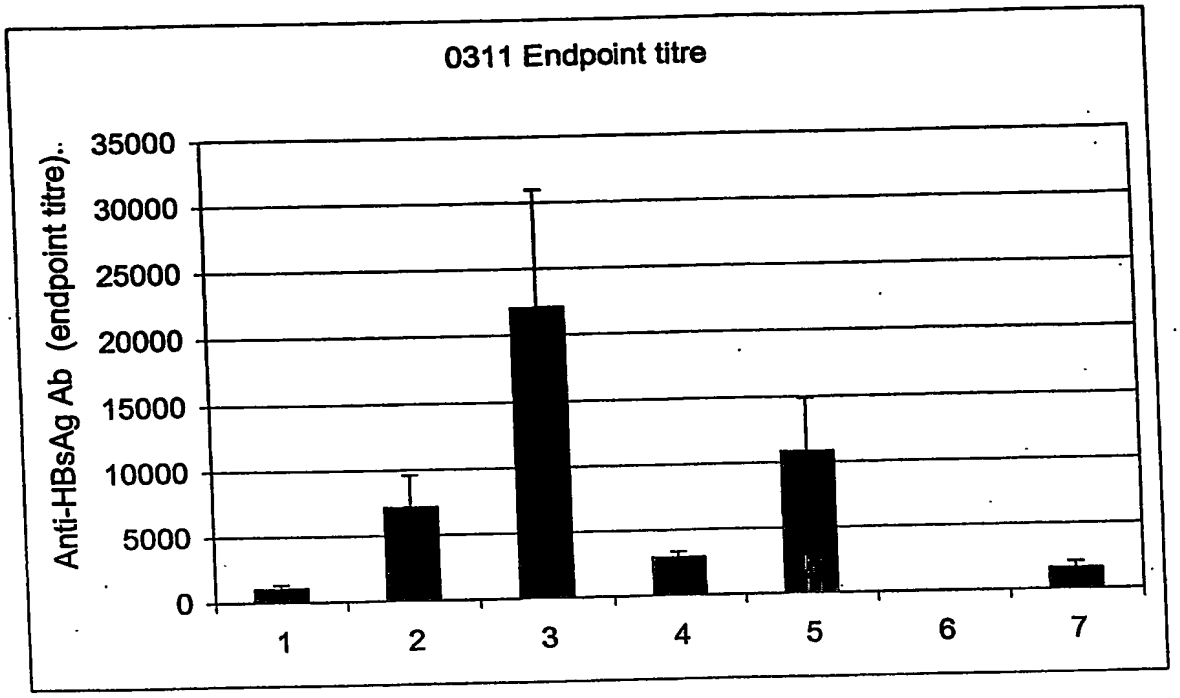

Figure 33

n=3-4 +/- SEM

PRIME	BOOST
1. DNA.HBs i.m. Engerix s.c.	MVA.HBs i.d. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d
4. DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
5. HBsAg + MVA.HBs mix i.d	HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.	MVA.HBs i.d
7. Engerix-B s.c.	Engerix-B s.c.
8. Naive	

5.2 T-cell Responses

5.2.1 Peptide Stimulated Splenocytes

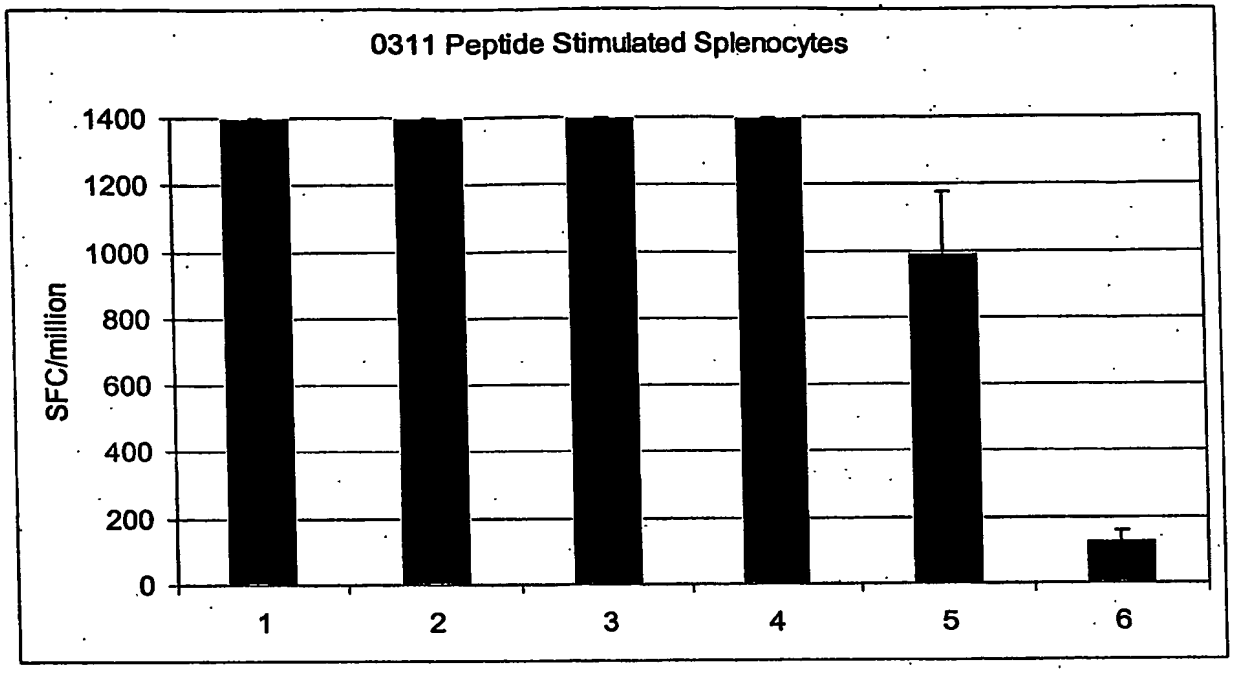

Figure 34

n=3-4 +/- SEM

PRIME	BOOST
1. DNA.HBs i.m. Engerix s.c.	MVA.HBs i.d. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d
4. DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
5. HBsAg + MVA.HBs mix i.d	HBsAg + MVA.HBs mix i.d
6. Engerix-B s.c.	Engerix-B s.c.

5.2.2 HBsAg stimulated splenocytes

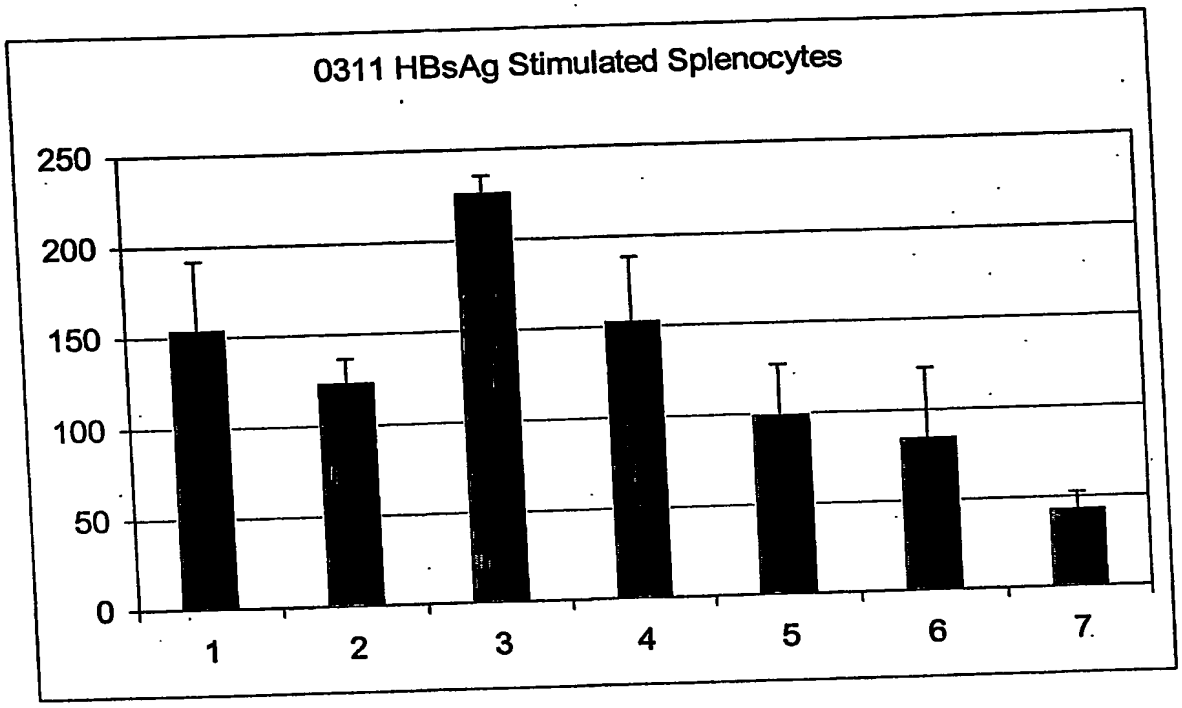

Figure 35
n=3-4 +/- SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

5.2.3 Peptide Stimulated Lymph Nodes

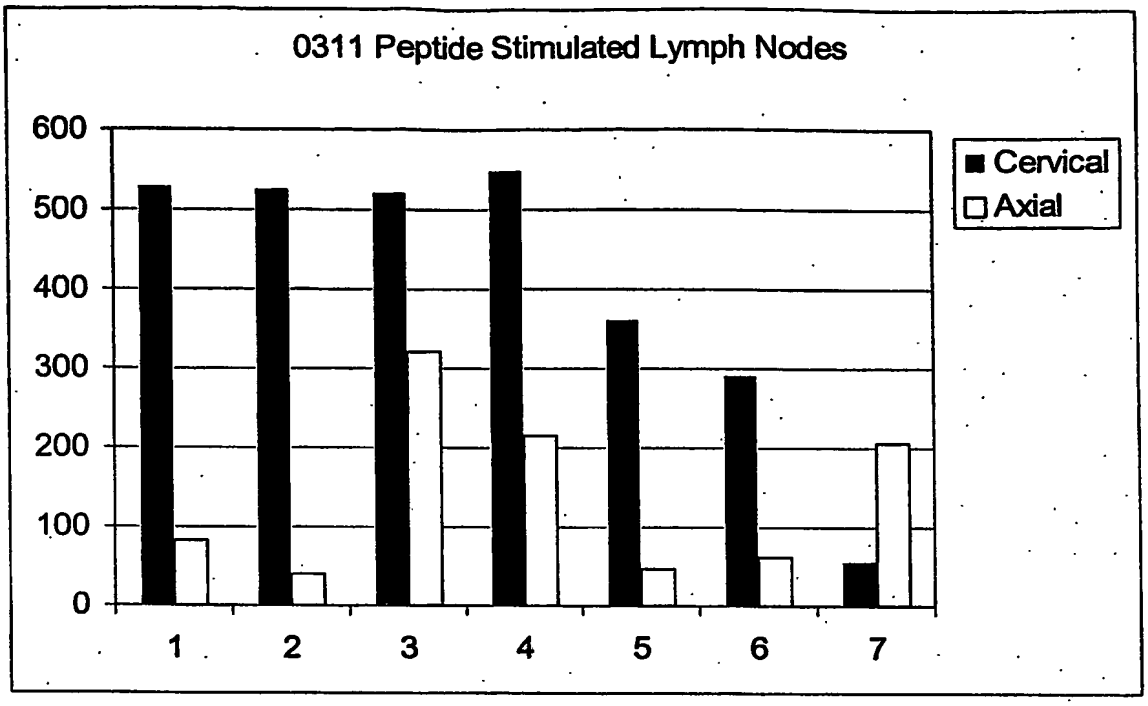

Figure 36
n=pooled lymph nodes from 3-4 animals

PRIME	BOOST
1. DNA.HBs i.m. Engerix s.c.	MVA.HBs i.d. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d
4. DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
5. HBsAg + MVA.HBs mix i.d	HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.	MVA.HBs i.d
7. Engerix-B s.c.	Engerix-B s.c.

5.2.4 HBsAg Stimulated Lymph Nodes

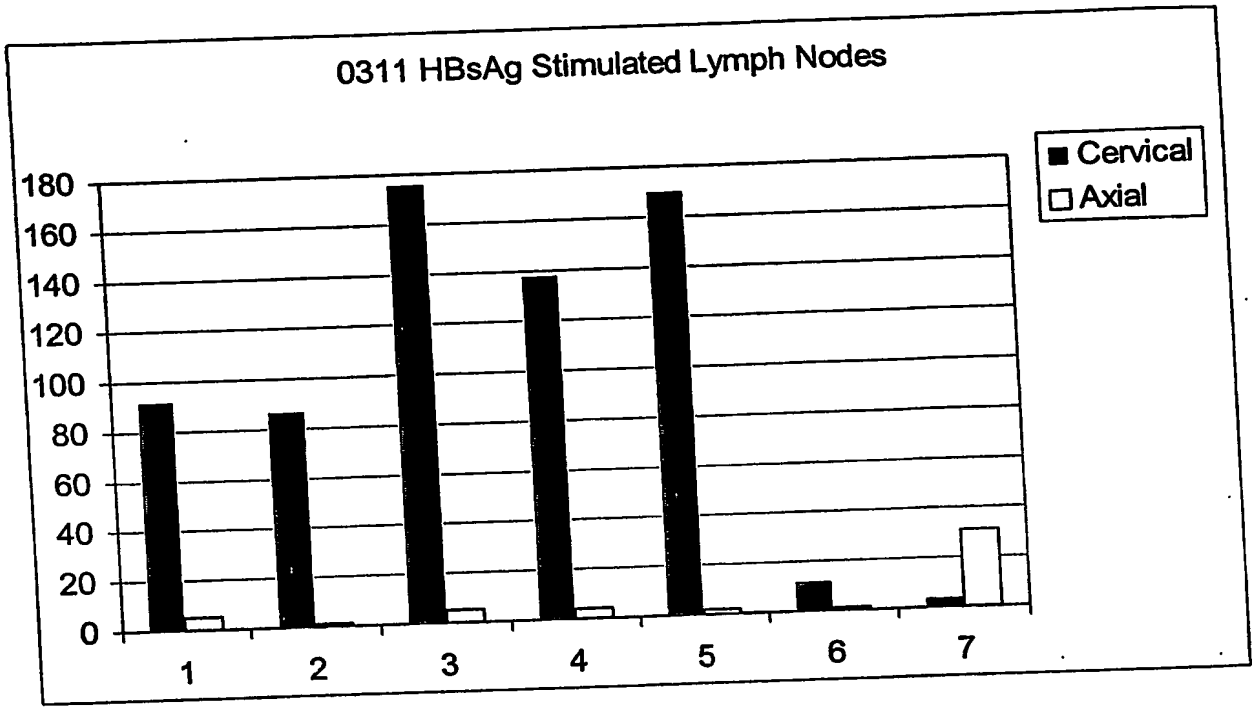

Figure 37
n=pooled lymph nodes from 3-4 animals

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

5.3 T-cell Responses in blood

5.3.1 0311b blood Elispot week 4

0311b was run concurrently with 0311 and will continue until week 12. Blood is taken for ELISA and blood Elispot weekly until sacrifice at week 12.

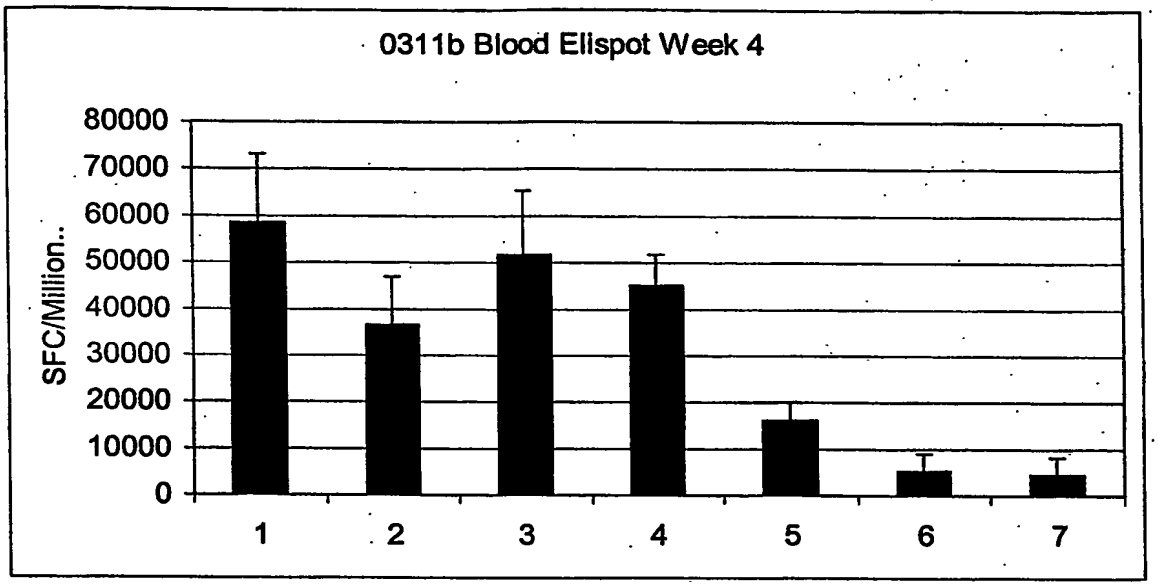

Figure 38
n= 4 +/- SEM

PRIME	BOOST
1. DNA.HBs i.m. Engerix s.c.	MVA.HBs i.d. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d
4. DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
5. HBsAg + MVA.HBs mix i.d	HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.	MVA.HBs i.d
7. Engerix-B s.c.	Engerix-B s.c.

Summary of responses to favourable regimes across experiments 2-5

Antibody Responses

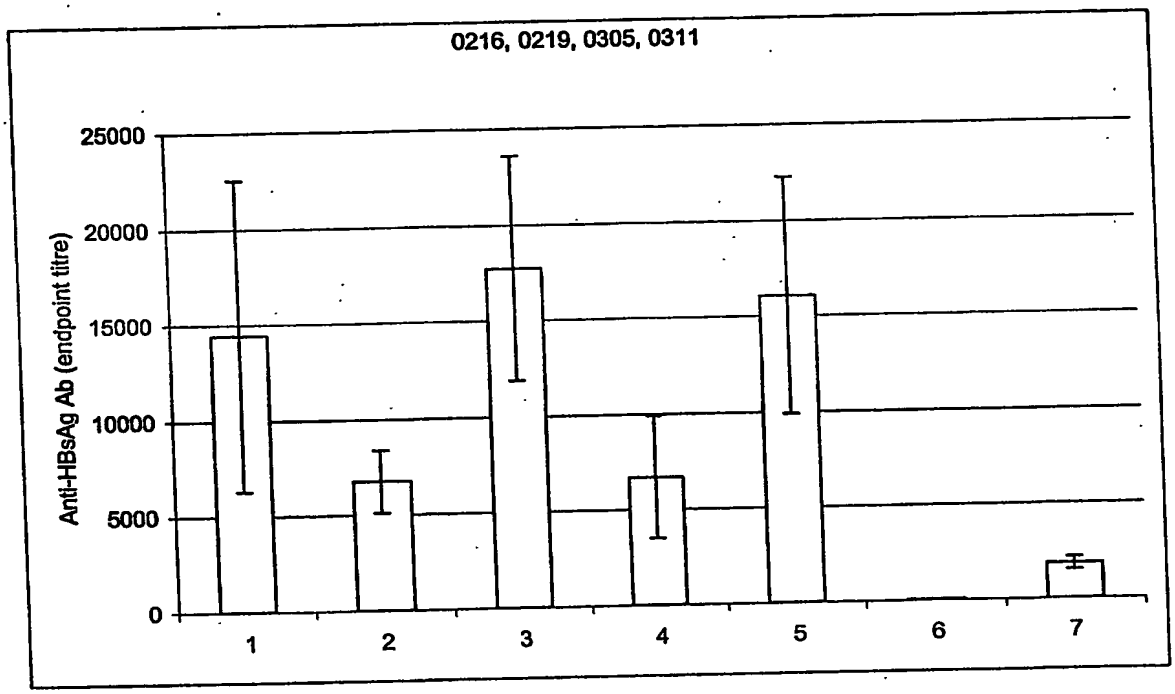

Figure 39
n=11-17 +/-SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

T-cell responses

Peptide Stimulated Splenocytes

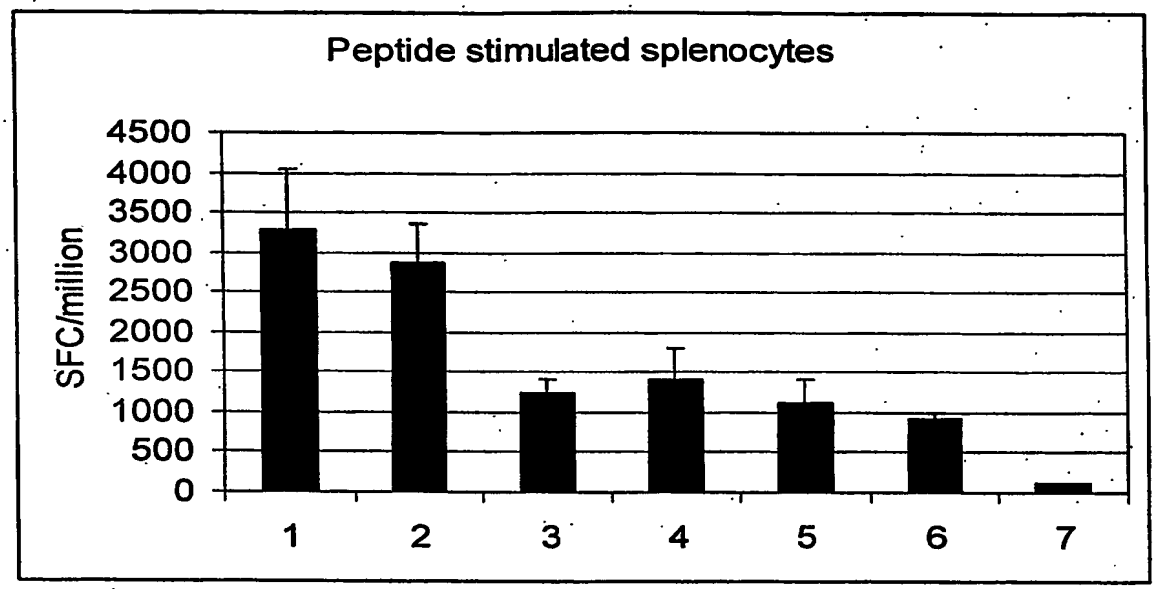

Figure 40
n=4-12 +/-SEM

PRIME	BOOST
1. DNA.HBs i.m. Engerix s.c.	MVA.HBs i.d. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.	HBsAg + MVA.HBs mix i.d
4. DNA.HBs i.m. & HBsAg i.d.	HBsAg + MVA.HBs mix i.d.
5. HBsAg + MVA.HBs mix i.d	HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.	MVA.HBs i.d
7. Engerix-B s.c.	Engerix-B s.c.

HBsAg Stimulated Splenocytes

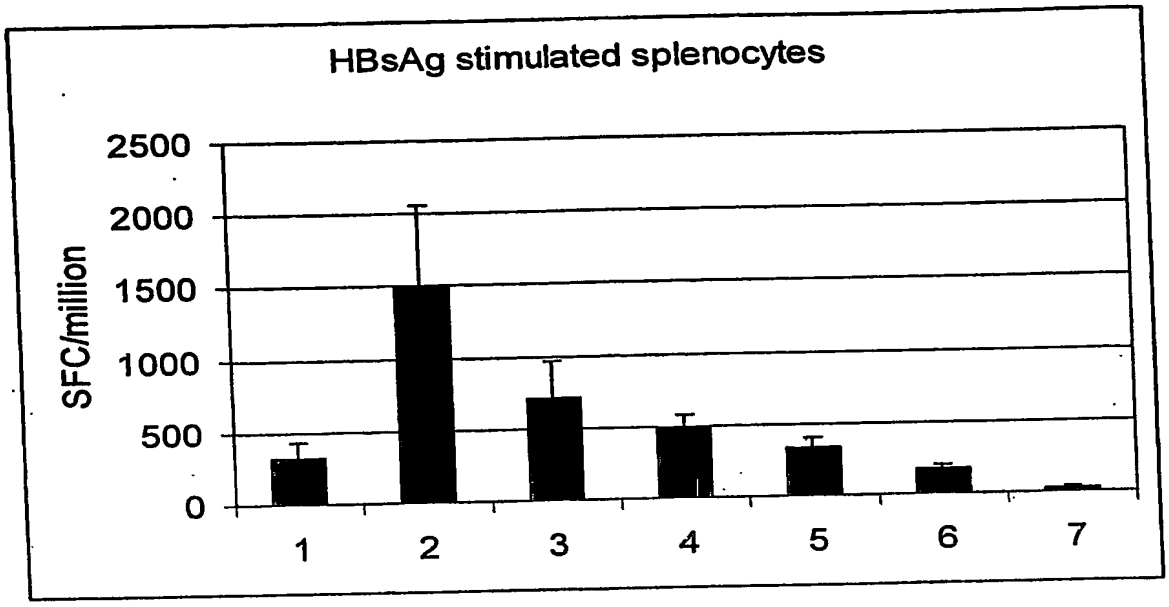

Figure 41
n=7-13 +/-SEM

PRIME
1. DNA.HBs i.m. Engerix s.c.
2. HBsAg + pSG2.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + FP9.LacZ 5 mix i.d.
4. DNA.HBs i.m. & HBsAg i.d.
5. HBsAg + MVA.HBs mix i.d
6. DNA.HBs i.m.
7. Engerix-B s.c.

BOOST
MVA.HBs i.d. Engerix s.c.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d
MVA.HBs i.d
Engerix-B s.c.

Experiment 6

Aim; to establish whether use of Adenovirus vectors boosts antibody and cellular responses to HBsAg. Furthermore, to establish whether heterologous immunisation induces strong cellular and humoral responses without DNA priming.

The CSP (circumsprozoite protein) from *Plasmodium berghei* (Pb) is used in some groups as the antigen, delivered using an Adenoviral vector.

6.1 Antibody Responses

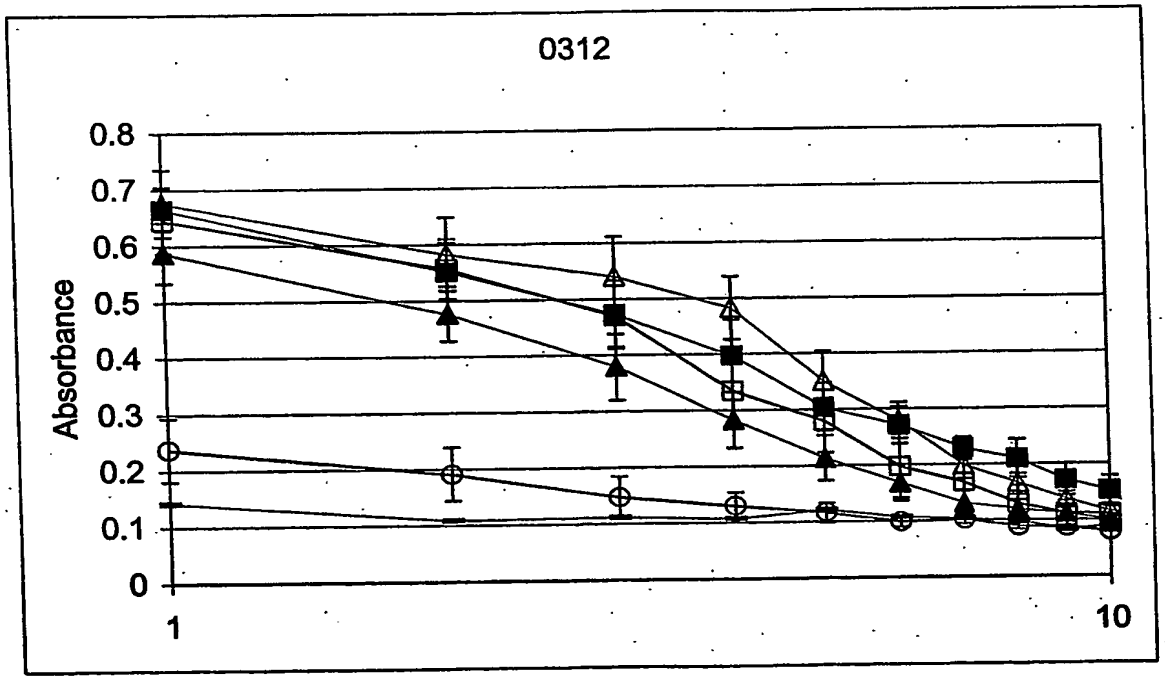

Figure 42

n=4 +/- SEM

PRIME
- Δ HBsAg + FP9.LacZ mix i.d.
- ▲ HBsAg + Adeno.PbCSP mix i.d.
- □ DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.
- ■ HBsAg + MVA.LacZ mix i.d.
- ○ DNA.HBs i.m. & Engerix-B s.c.
- — Naive

BOOST
- HBsAg + MVA.HBs mix i.d.
- HBsAg + MVA.HBs mix i.d.
- HBsAg + MVA.HBs mix i.d.
- HBsAg + MVA.HBs mix i.d.
- MVA.HBs s.c.

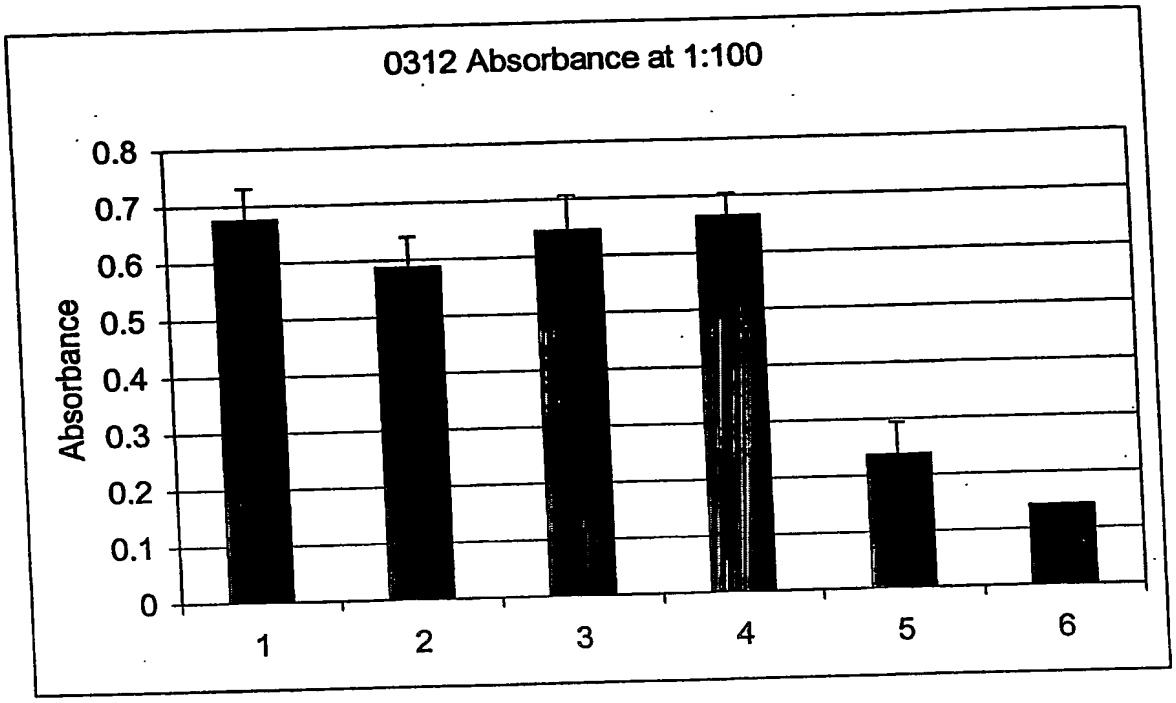

Figure 43

n=4 +/- SEM

PRIME

1. HBsAg + FP9.LacZ mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.
4. HBsAg + MVA.LacZ mix i.d.
5. DNA.HBs I.m. & Engerix-B s.c.
6. Naive

BOOST

HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
MVA.HBs s.c.

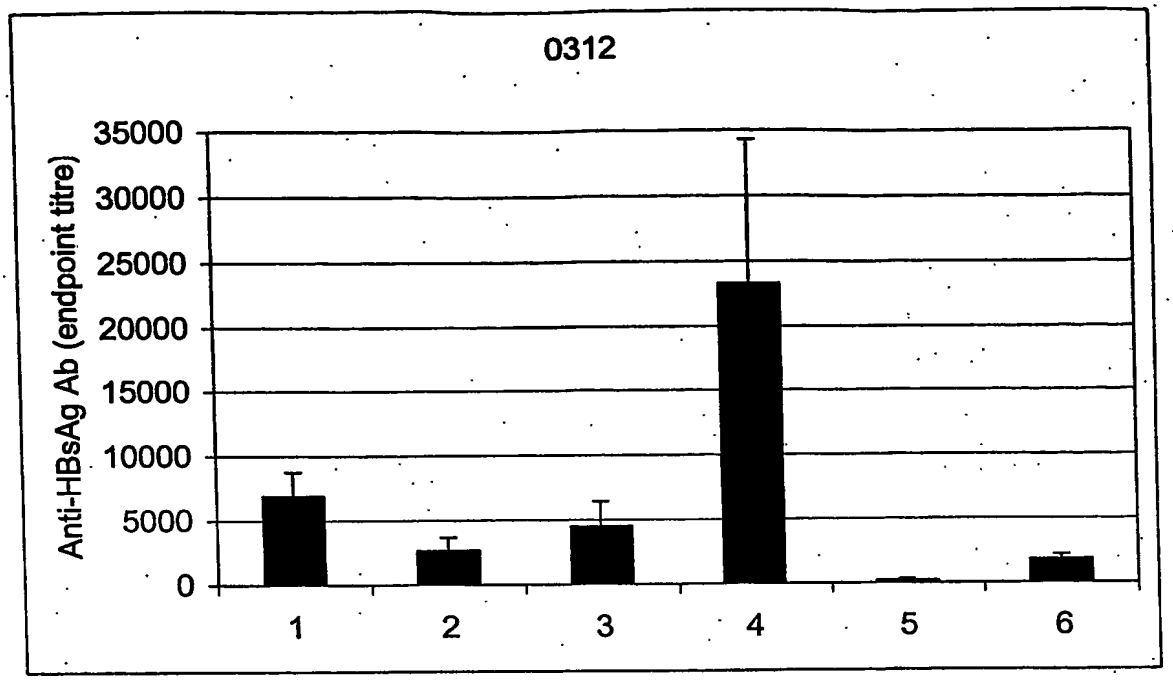

Figure 44

n=4 +/- SEM
(except group 6 where n=16)

PRIME	BOOST
1. HBsAg + FP9.LacZ mix i.d.	HBsAg + MVA.HBs mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.	HBsAg + MVA.HBs mix i.d.
4. HBsAg + MVA.LacZ mix i.d.	HBsAg + MVA.HBs mix i.d.
5. DNA.HBs i.m. & Engerix-B s.c.	MVA.HBs s.c.
6. Engerix-B s.c.	Engerix-B s.c. (exp. 2-5)

6.2 T-Cell Responses

6.2.1 Peptide Stimulated Splenocytes

for groups 2 and 3, the CSP epitope was varied between the IPQ epitope and the Pb9 epitope

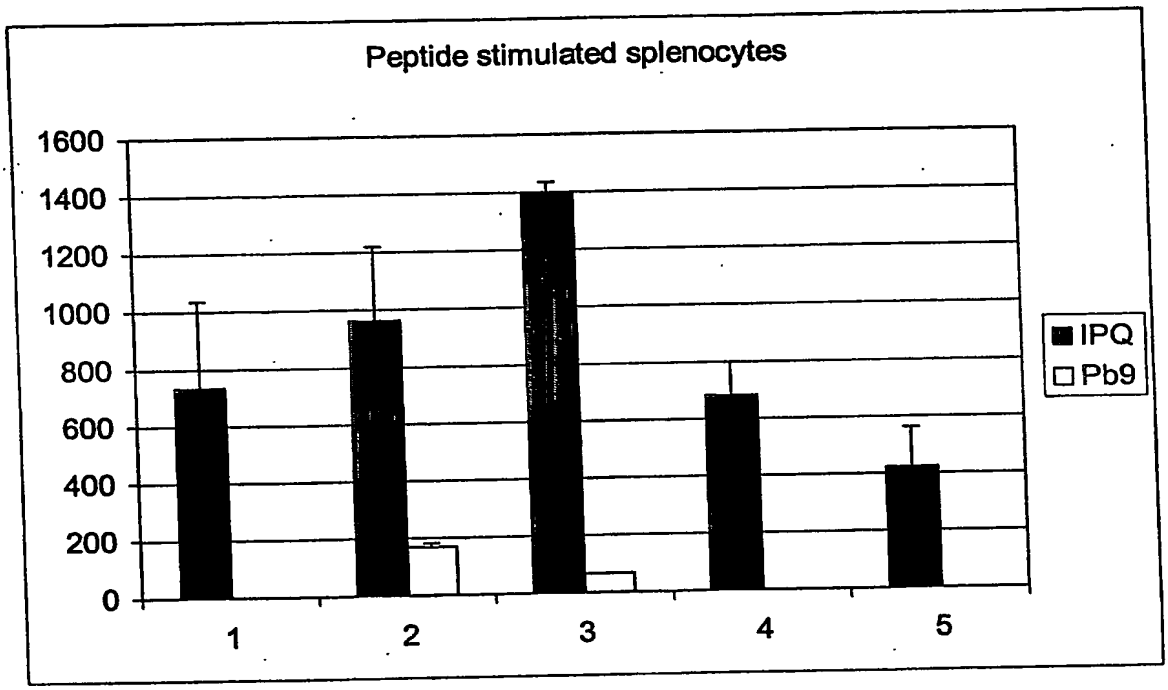

Figure 45

n=4 +/- SEM

PRIME

1. HBsAg + FP9.LacZ mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.
4. HBsAg + MVA.LacZ mix i.d.
5. DNA.HBs i.m. & Engerix-B s.c.

BOOST

HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
HBsAg + MVA.HBs mix i.d.
MVA.HBs s.c.

6.2.2 HBsAg Stimulated Splenocytes

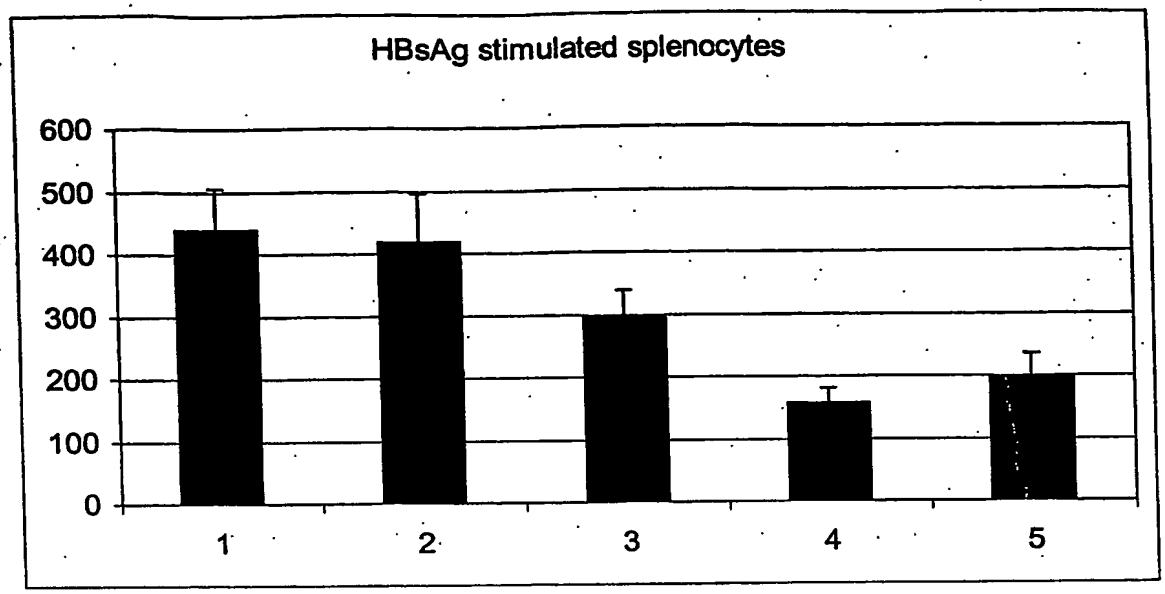

Figure 46

n=4 +/- SEM

PRIME	BOOST
1. HBsAg + FP9.LacZ mix i.d.	HBsAg + MVA.HBs mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.	HBsAg + MVA.HBs mix i.d.
4. HBsAg + MVA.LacZ mix i.d.	HBsAg + MVA.HBs mix i.d.
5. DNA.HBs i.m. & Engerix-B s.c.	MVA.HBs s.c.

6.2.3 Peptide Stimulated Lymph Nodes

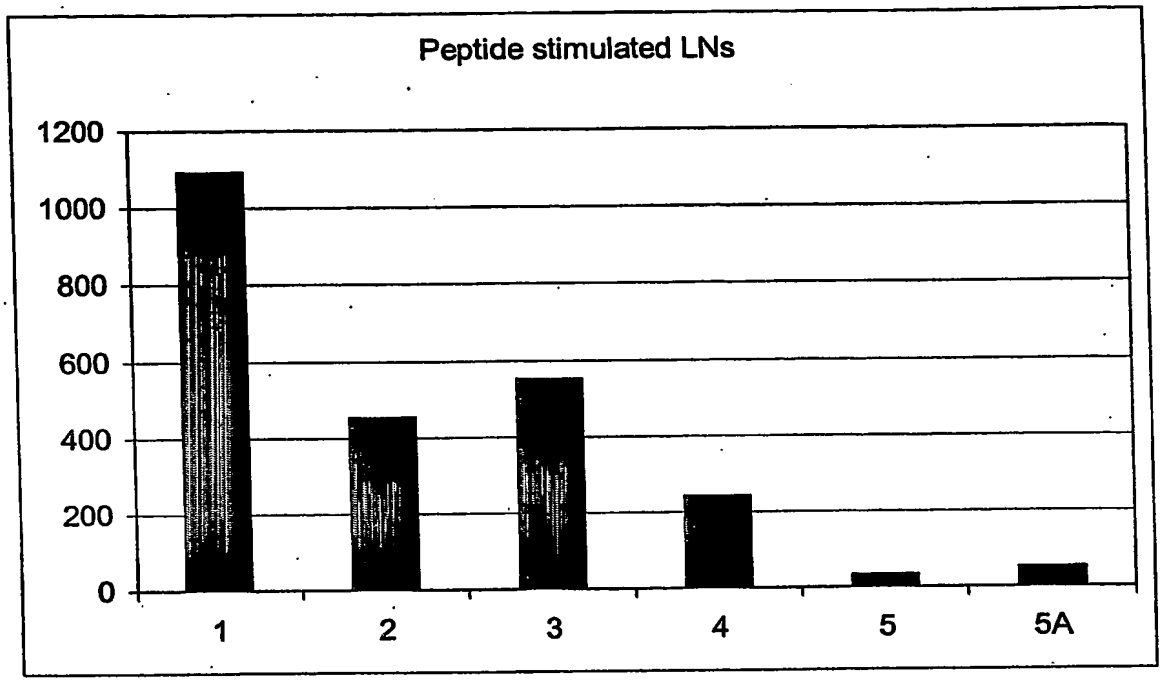

Figure 47

n=pooled cervical lymph nodes from 4 animals (except 5A where lymph nodes were axial)

PRIME	BOOST
1. HBsAg + FP9.LacZ mix i.d.	HBsAg + MVA.HBs mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.	HBsAg + MVA.HBs mix i.d.
4. HBsAg + MVA.LacZ mix i.d.	HBsAg + MVA.HBs mix i.d.
5. DNA.HBs i.m. & Engerix-B s.c.	MVA.HBs s.c.
5A. DNA.HBs i.m. & Engerix-B s.c.	MVA.HBs s.c.

6.2.4 HBsAg Stimulated Lymph Nodes

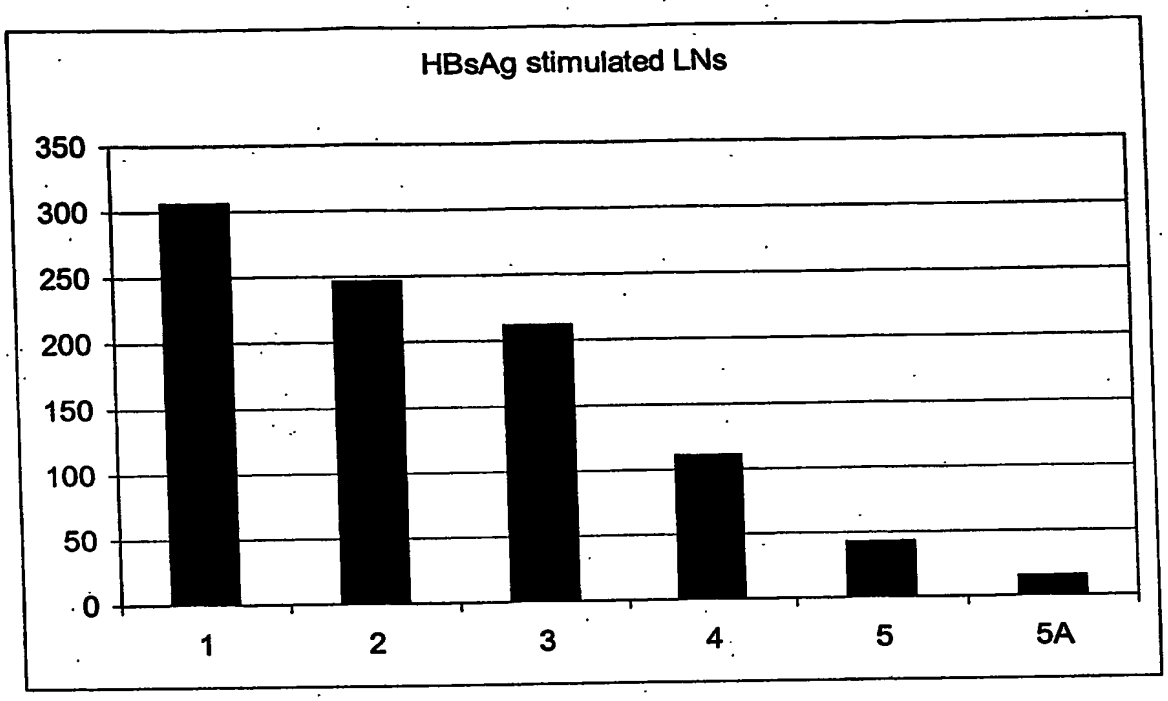

Figure 48

n=pooled cervical lymph nodes from 4 animals (except 5A where lymph nodes were axial)

PRIME	BOOST
1. HBsAg + FP9.LacZ mix i.d.	HBsAg + MVA.HBs mix i.d.
2. HBsAg + Adeno.PbCSP mix i.d.	HBsAg + MVA.HBs mix i.d.
3. DNA.HBs i.m. & HBsAg + Adeno.PbCSP mix i.d.	HBsAg + MVA.HBs mix i.d.
4. HBsAg + MVA.LacZ mix i.d.	HBsAg + MVA.HBs mix i.d.
5. DNA.HBs i.m. & Engerix-B s.c.	MVA.HBs s.c.
5A. DNA.HBs i.m. & Engerix-B s.c.	MVA.HBs s.c.

Experiment 7

Homologous immunization with HBsAg mixed with non-recombinant MVA, NYVAC, ALVAC, FP or ADV. The non-recombinant poxviruses MVA(MVAnr), FP(FPnr), ALVAC(ALVACnr) and NYVAC(NYVACnr) adjuvant potent antibody responses when co-administered i.d. (intradermally) twice with rHBsAg.

All poxviruses elicited specific antibodies against rHBsAg whereas ADVnr failed to increase antibody responses above the level of repeat rHBsAg immunization (Fig. 49). The attenuated vaccinia virus, NYVACnr, induced similar antibody responses to MVAnr while the avipox virus, ALVACnr elicited the highest levels of anti-rHBsAg antibodies. All viruses increased cellular responses compared to repeat immunization with rHBsAg (Fig. 50 & 51). The most significant increase in T cell responses compared to rHBsAg immunization were induced by ALVACnr (peptide: $p=0.029$, rHBsAg: $p=0.021$). However, non-recombinant MVA, FP, NYVAC and ADV also significantly increased T cell responses compared to rHBsAg immunization (Peptide: $p=0.017$, 0.021, 0.021, 0.021, rHBsAg: $p=0.006$, 0.042, 0.021, 0.018). All viruses induced potent responses to peptide in facial DLN (Draining Lymph Nodes) although, surprisingly, the most potent responses were induced by ALVACnr (Fig. 52 & 53).

Homologous immunization with non-recombinant poxvirus mixed with antigen is an efficient method for inducing potent antibody and cellular responses against the co-administered protein. It can be seen here that although adenovirus is effective, poxvirus is preferred.

Co-administration of a non-recombinant or "empty" viral vector, that does not encode an antigen, together with an antigen induces increased antibody and T cell responses compared to the antigen alone. In particular, it can be seen that a homologous prime boost regimen is preferable and, furthermore, that ALVAC is particularly effective as the vector.

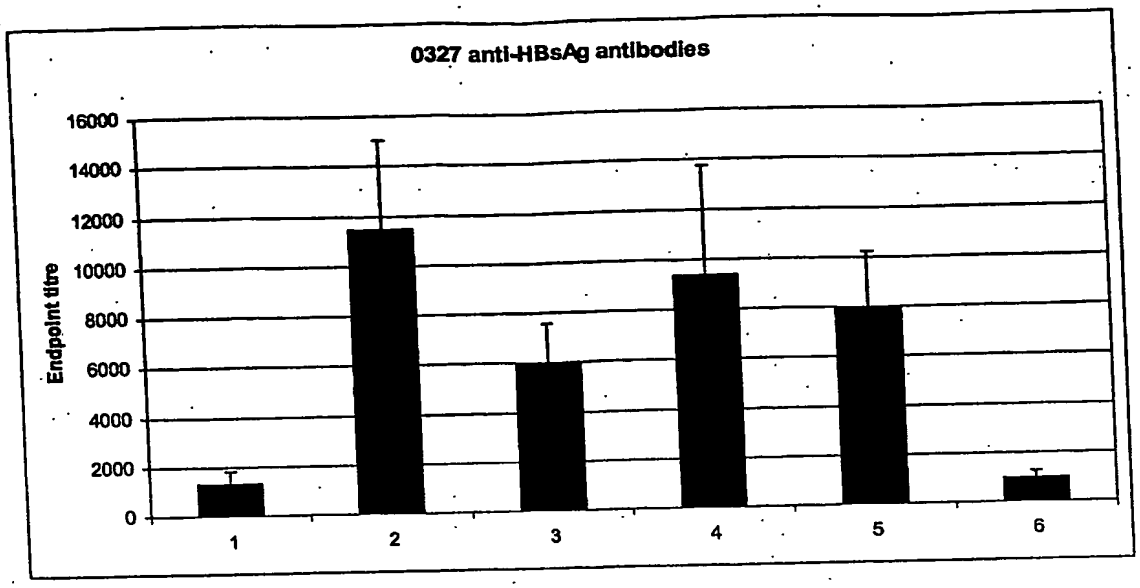

Figure 49

1. [Adeno.LacZ + Ag] id x 2
2. [ALVAC+ Ag] id x 2
3. [FP.LacZ + Ag] id x 2
4. [MVA.LacZ + Ag] id x 2
5. [NYVAC.LacZ + Ag] id x 2
6. 2 x Ag id

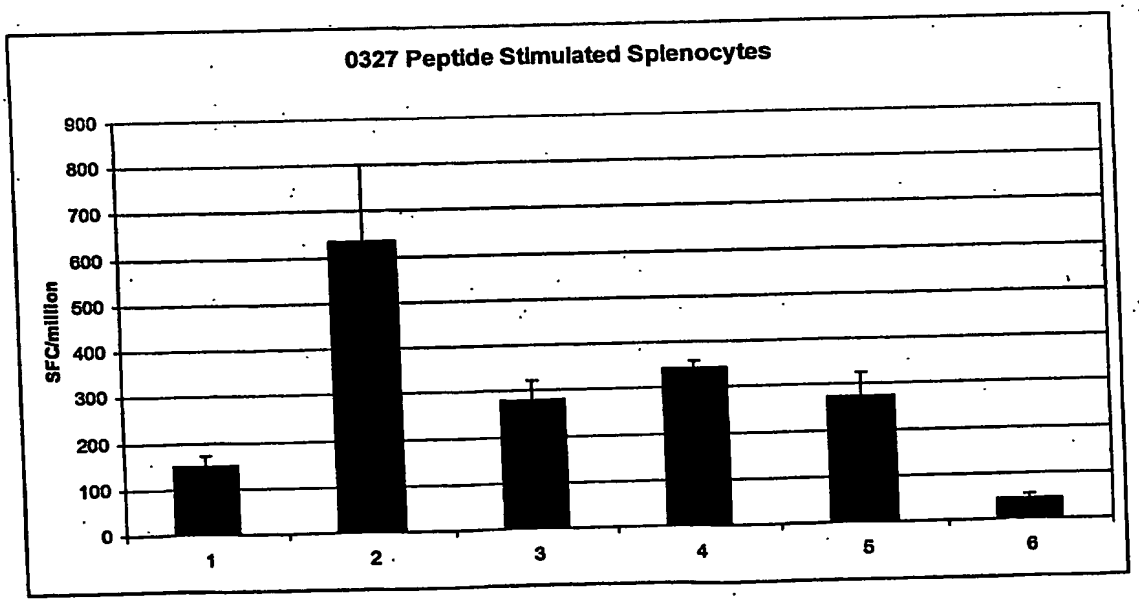

Figure 50

1. [Adeno.LacZ + Ag] id x 2
2. [ALVAC+ Ag] id x 2
3. [FP.LacZ + Ag] id x 2
4. [MVA.LacZ + Ag] id x 2
5. [NYVAC.LacZ + Ag] id x 2
6. 2 x Ag id

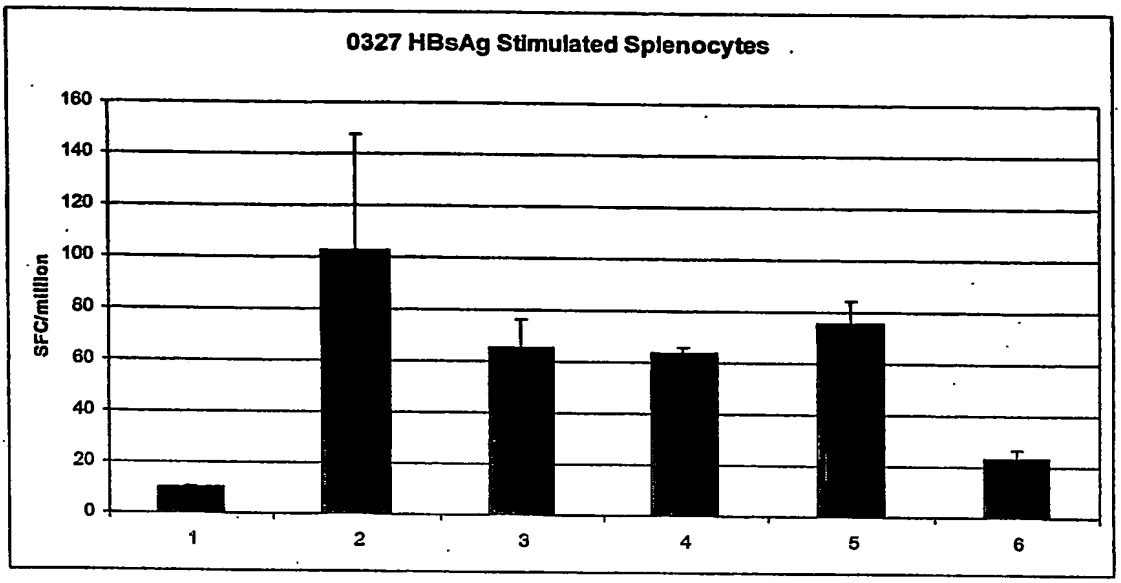

Figure 51

1. [Adeno.LacZ + Ag] id x 2 2. [ALVAC+ Ag] id x 2
3. [FP.LacZ + Ag] id x 2 4. [MVA.LacZ + Ag] id x 2
5. [NYVAC + Ag] id x 2 6. 2 x Ag id

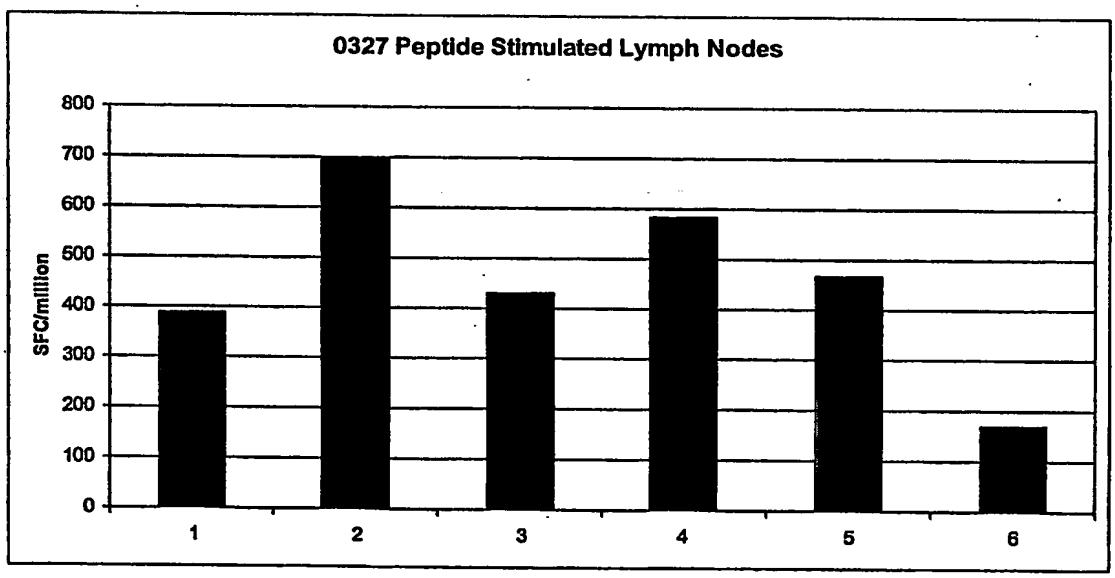

Figure 52

1. [Adeno.LacZ + Ag] id x 2 2. [ALVAC+ Ag] id x 2
3. [FP.LacZ + Ag] id x 2 4. [MVA.LacZ + Ag] id x 2
5. [NYVAC + Ag] id x 2 6. 2 x Ag id

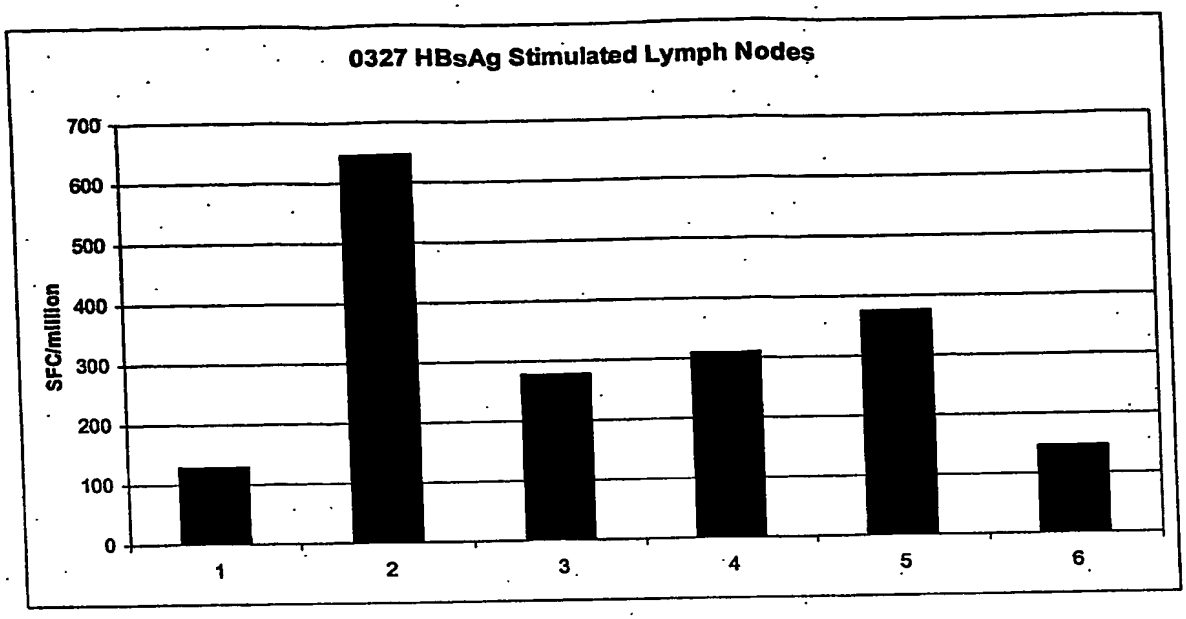

Figure 53

1. [Adeno.LacZ + Ag] id x 2
2. [ALVAC+ Ag] id x 2
3. [FP.LacZ + Ag] id x 2
4. [MVA.LacZ + Ag] id x 2
5. [NYVAC + Ag] id x 2
6. 2 x Ag id

Experiment 8 Longevity of favourable regimes in 0311b

Favourable antibody and cellular inducing regimes from experiments 1-4 were analysed from day 0 (day of prime) until day 148 for induction of antibodies to HBsAg and day 216 for cellular response to peptide.

Immunisation with DNA i.m. [FP+Ag] i.d./[MVA.HBs+Ag]i.d. induced the most potent and long-lived responses to HBsAg although all antibody levels in all other regimens except DNAi.m.Ag i.d./[MVA.HBs+Ag]i.d. were consistently higher than repeat Engerix-B immunization until day 120 when all responses were in decline (Fig. 54).

All regimens induced much greater $CD8^+$ T cell responses to peptide when compared to repeat Engerix-B immunization (Fig. 55). Immunisation with DNA i.m. Engerix-B s.c./MVA.Hbs i.d. Engerix-B s.c. induced higher T cell responses to peptide than other regimens until day 120 post-prime. These results demonstrate the longevity of responses induced by the combination of T cell and antibody inducing vaccines.

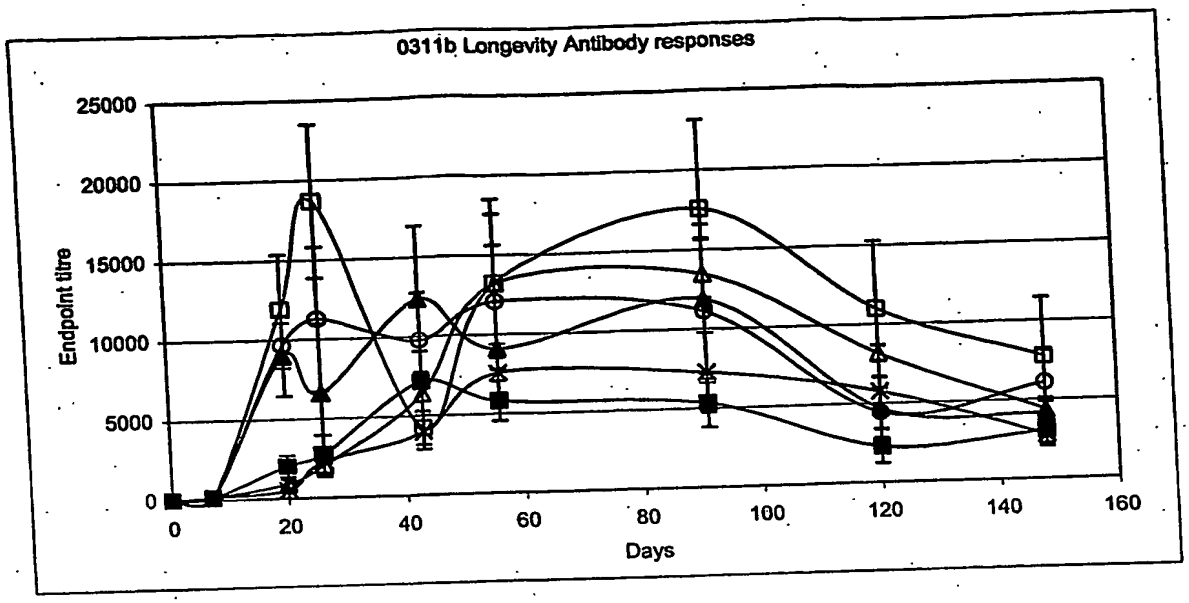

Figure 54

△DNAimEngsc/MVA.HbsidEngsc

□DNA-im[FP+Ag]id/[MVA.HBs+Ag]id

○ [MVA.HBs+Ag]id/[MVA.HBs+Ag]id

▲ [DNA+Ag]id/[MVA.HBs+Ag]id

■DNAimAgId/[MVA.HBs+Ag]Id

∗ Engsc/Engsc

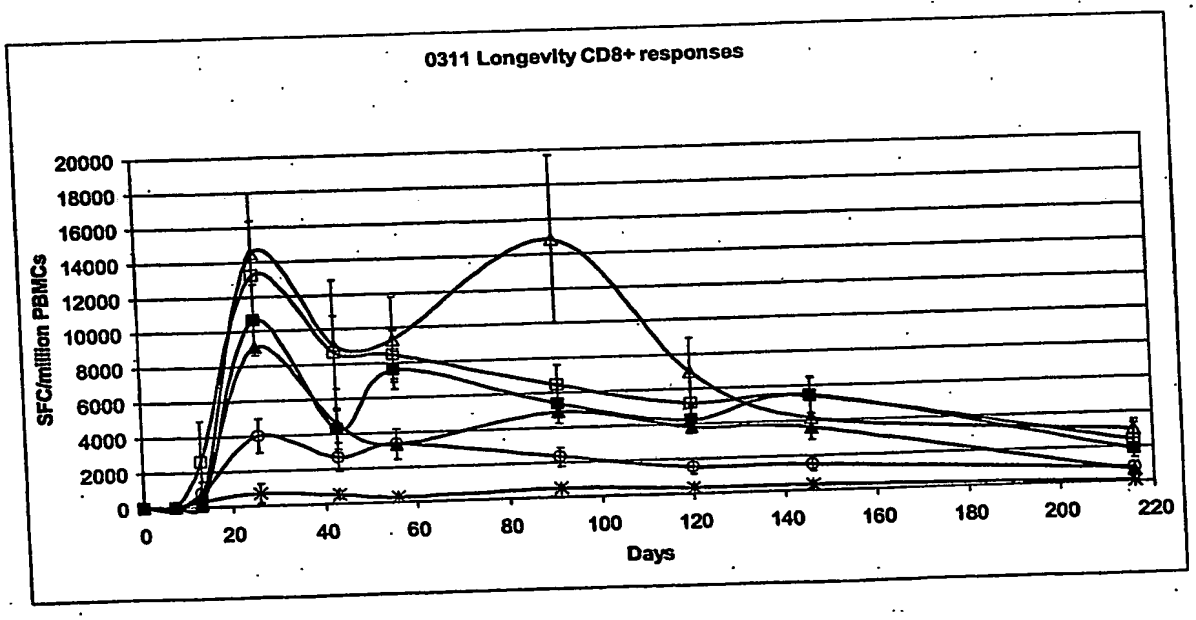

Figure 55

△DNAimEngsc/MVA.HbsidEngsc

□DNA-im[FP+Ag]id/[MVA.HBs+Ag]id

○ [MVA.HBs+Ag]id/[MVA.HBs+Ag]id

▲ [DNA+Ag]id/[MVA.HBs+Ag]id

■DNAimAgId/[MVA.HBs+Ag]Id

∗ Engsc/Engsc

Experiment 9 Further ELISA data for experiments 1-7)

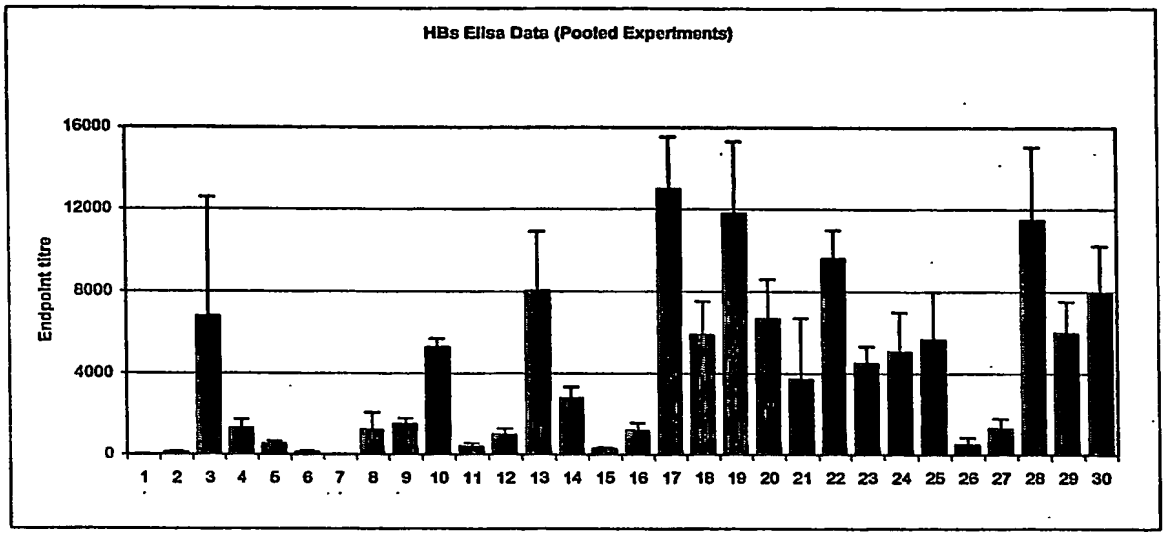

Figure 56

1. DNA/MVA *iv*
2. NIL/Eng *sc*
3. DNA/[MVA+Eng] *sc*
4. DNA/Mva *iv* Eng *sc*
5. DNA/Eng *sc*
6. DNA/(MVA+Al)*sc*
7. DNA/MVA *id*
8. DNA*im* Eng*sc* / [MVA+Eng]*sc*
9. DNA*im* Eng*sc* / MVA*id* Eng*sc n=15*
10. DNA*im* Eng*sc*/ [MVA.Lac+Eng]*sc*
11. 2 x Ag *sc*
12. 2 x Ag *id*
13. 2 x [MVA.Lac+Ag] *id*
14. 2 x Eng *sc* *n=12*
15. 2 x MVA.Lac *id* +Eng *sc*
16. 2 x [MVA.LacZ+Eng-B] s.c.
17. 2 x [MVA+Ag]*id* *n=8*
18. [DNA+Ag]*id* / [MVA+Ag]*id*
19. DNA*im* [FP9.Lac+Ag]*id* / [MVA+Ag]*id*
20. DNA*im* Ag*id* / [MVA+Ag]*id*
21. [MVA.Lac+Ag] *id* / [FP.Lac+Ag]*id*
22. [FP9.Lac+Ag]*id* / [MVA+Ag]*id*
23. [AD.CSP+Ag]*id* / [MVA+Ag]*id*
24. DNA *im* [AD.CSP+Ag]*id* / [MVA+Ag]*id*
25. [MVA.Lac+Ag]*id* / [MVA+Ag]*id*
26. DNA*im* Eng*sc* / MVA *sc*
27. [Adeno.LacZ + Ag] id x 2
28. [ALVAC+ Ag] id x 2
29. [FP.LacZ + Ag] id x 2
30. [NYVAC + Ag] id x 2

Experiment 10 viral modulation of IgG subclasses

The ratio of isotype subclasses IgG1 and IgG2a gives an indication of Th2 or Th1 bias of humoral responses respectively. The effect of DNA, poxviruses and ADV on IgG subclass division was determined (Fig. 57) by measuring IgG subclasses / isotypes using biotin conjugated anti-mouse IgG1 or IgG2a antibody (Pharmingen) followed by incubation with ExtrAvidin (Sigma) for isotype analysis.

Engerix-B predominantly induced IgG1 antibodies which is indicative of a Th2 biased humoral response. This concurs with the poor IFN-γ production by peptide and rHBsAg stimulated splenocytes from Engerix-B immunized animals (figure 15, group 4 and figure 16, group 4).

Priming with a mixture of rHBsAg and recombinant MVA or non-recombinant FP, MVA, ALVAC, NYVAC with or without DNA induced equal levels of IgG1/IgG2a or higher IgG2a. Surprisingly, homologous immunization with rHBsAg and the avipox viruses ALVACnr and FPnr consistently induced a higher level of IgG2a than IgG1. In contrast, combination of rHBsAg with non-recombinant MVA and NYVAC, both derived from vaccinia, induced a 1:1 ratio of IgG1:IgG2a. Absence of poxvirus in the prime predominantly lead to increased levels of IgG1 compared to IgG2a except in DNA/MVA where low levels of both IgG1:IgG2a were observed.

Conversely, co-administration of ADVnr induced higher IgG1 than IgG2a similar to responses following repeat immunization with rHBsAg alone, indicating a lack of adjuvant activity or antibody response of ADVnr.

The increased levels of IgG2a induced by poxviral priming indicates a Th1 biased humoral response, while priming with combinations of DNA, Engerix-B and rHBsAg lead to a higher ratio of IgG1 and therefore Th2 bias. Recombinant MVA, MVAnr and NYVACnr induced an even ratio of IgG1:IgG2a whereas FP and ALVAC skew the

response to IgG2a . These results indicated that equal Th-1 and Th-2 or biased responses could be primed to the same antigen depending on the co-delivered viral vector.

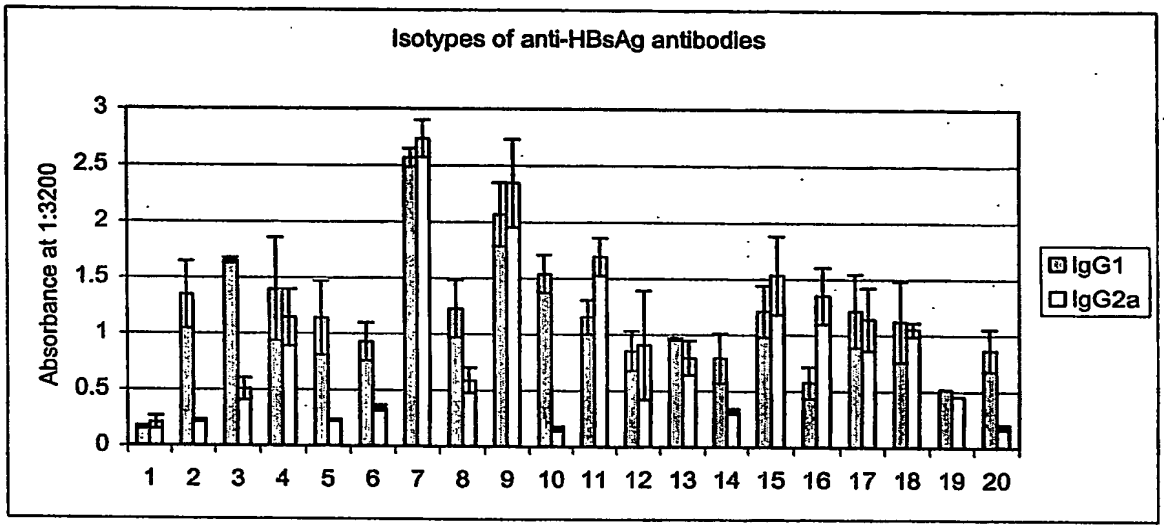

Figure 57

1. DNA/MVA *id*
2. 2.DNA*im* Eng*sc* / MVA*id* Eng*sc*
3. [DNA+Ag]*id* / [MVA+Ag]*id*
4. DNA*im* Ag*id* / [MVA+Ag]*id*
5. DNA*im* Eng*sc* / MVA*id* Eng*sc*
6. [DNA+Ag]*id* / [MVA+Ag]*id*
7. DNA*im* [FP9.Lac+Ag]*id* / [MVA+Ag]*id*
8. DNA*im* Ag *id* / [MVA+Ag]*id*
9. 2 x [MVA+Ag]*id*
10. 2 x Eng *sc*
11. [FP9.Lac+Ag]*id* / [MVA+Ag]*id*
12. [AD.CSP+Ag]*id* / [MVA+Ag]*id*
13. DNA *im* [AD.CSP+Ag]*id* / [MVA+Ag]*id*
14. [Adeno.LacZ + Ag] id x 2
15. [ALVAC+ Ag] id x 2
16. [FP.LacZ + Ag] id x 2
17. [MVA.LacZ + Ag] id x 2
18. [NYVAC.LacZ + Ag] id x 2
19. 2 x Ag *sc*
20. 2 x Ag *id*

Experiment 11 : *P berghei* murine model

This investigated the *P berghei* murine model of malaria. As with the Hepatitis B model in previous experiments, this experiment combines a T-cell inducing vaccine with an antibody inducing vaccine leading to potent induction of both responses concurrently.

Mice were primed at day 0 and boosted on day 22. T cell responses to a dominant $CD8^+$ epitope of CSP (Pb9) were measured in blood on day 21 and 36. Antibody responses to the B-cell epitope (DP_4NPN) present in both the Alum–precipitated vaccine (Apv) and CSP encoded by DNA, MVA and FP as well as HBc responses were measured on days 14 and 37. The terms AP and Apv are used interchangeably.

No significant levels of antibodies to DP_4NPN or HBc in any vaccine regimen were detected at day 14. The vaccine regimens of PBS x 2, DNA i.m./MVA i.d. and MVA i.d./FP i.d. also failed to induce detectable levels of anti-DP_4NPN antibodies on day 37 (Fig.58). Repeat immunization with 5μg of Apv i.d. and 10μg of Apv i.p. strongly induced antibodies to DP_4NPN although i.d. immunization induced slightly higher responses. Conversely, the immunization regimens of DNA i.m. + AP i.p./ MVA+AP i.p. and FP i.d. + AP i.p./MVA i.d. Ap i.p. reduced antibody levels compared to repeat Apv immunization. However the regimen FP+AP i.d./MVA+AP i.d., where FP and MVA were mixed with Apv and administered i.d., greatly enhanced antibody responses to DP_4NPN compared to repeat Apv immunization.

Immunization with DNA i.m./MVA i.d. and FP i.d./MVA i.d. both induce high levels of peptide specific T cells although FP/MVA induced a greater level than DNA/MVA (Fig. 59). Repeat immunisation with Apv i.d. or i.p. failed to induce any T cell response to peptide above background. Combination of DNA/MVA with Apv (DNA i.m. + AP i.p./ MVA+AP i.p.) lead to a great increase in T cell responses to peptide compared to DNA/MVA immunisation. Concurrent immunization with FP/MVA and Apv at separate sites (FP i.d. + AP i.p./MVA i.d. Ap i.p.) induced similar levels of

peptide specific T cells to FP/MVA immunization. However, if Apv was mixed with FP/MVA and administered i.d. (FP+AP i.d./MVA+AP i.d.), T cell responses to peptide were reduced when compared to immunization with FP/MVA alone.

Stimulation of PBMCs with HBc induced minimal responses in all regimens except FP+AP i.d./MVA+AP i.d. where these responses were clearly amplified (Fig. 60). This increase in T cells specific for HBc may represent both $CD4^+$ and $CD8^+$ populations and are an example of the capability of FP and MVA to adjuvant a protein that is not encoded within either virus. It also illustrates the virus' ability to adjuvant T cell responses in the presence of alum. This is surprising, as the presence of alum would have been expected to negate or at least counteract any T cell response.

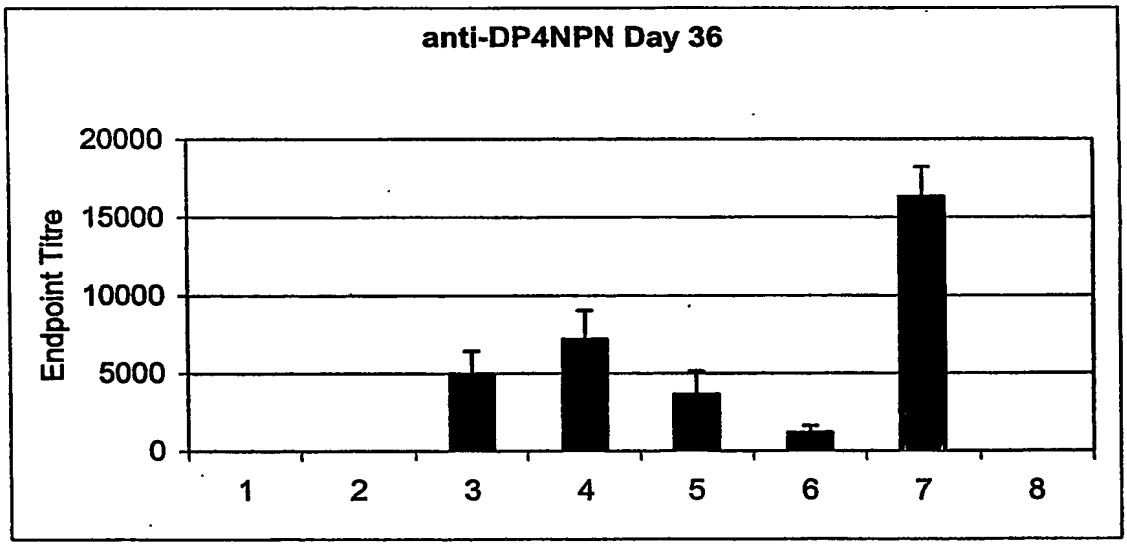

Figure 58

1. FP/MVA i.d.
2. DNA i.m./MVA i.d.
3. AP i.p. x 2 (10ug)
4. AP i.d. x 2 (5ug)
5. DNA i.m. + AP i.p./ MVA+AP i.p.
6. FP i.d. + AP i.p./MVA i.d. Ap i.p.
7. FP+AP i.d./MVA+AP i.d.
8. PBS x 2

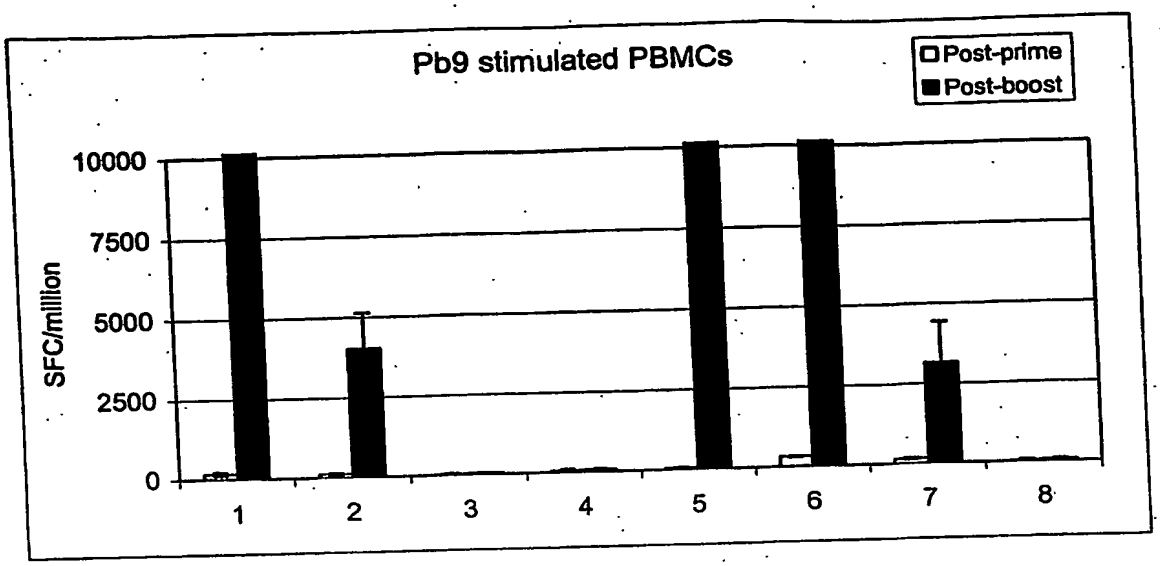

Figure 59

1. FP/MVA i.d.
2. DNA i.m./MVA i.d.
3. AP i.p. x 2 (10ug)
4. AP i.d. x 2 (5ug)
5. DNA i.m. + AP i.p./ MVA+AP i.p.
6. FP i.d. + AP i.p./MVA i.d. Ap i.p.
7. FP+AP i.d./MVA+AP i.d.
8. PBS x 2

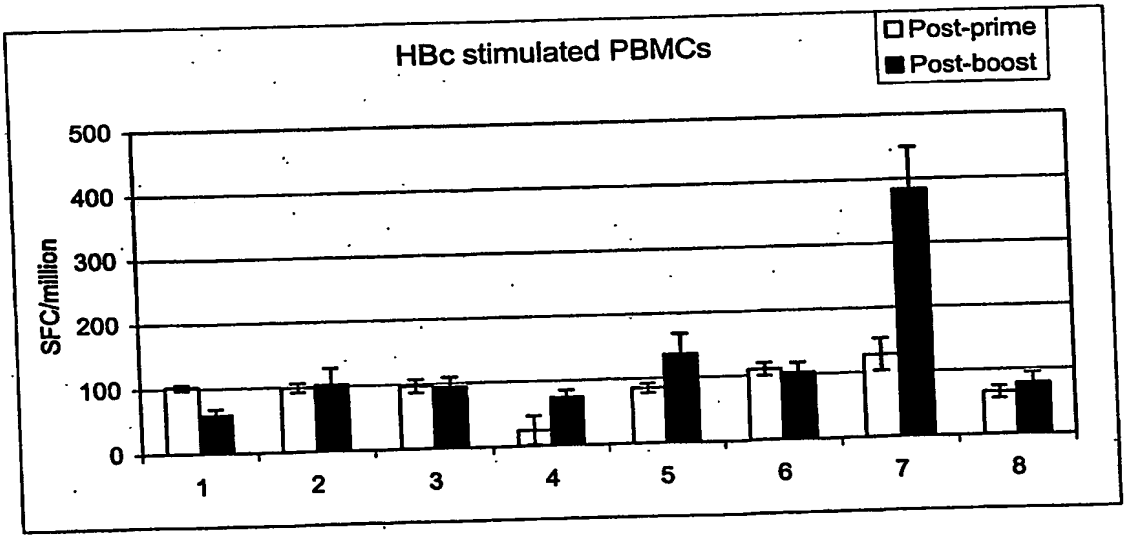

Figure 60

1. FP/MVA i.d.
2. DNA i.m./MVA i.d.
3. AP i.p. x 2 (10ug)
4. AP i.d. x 2 (5ug)
5. DNA i.m. + AP i.p./ MVA+AP i.p.
6. FP i.d. + AP i.p./MVA i.d. Ap i.p.
7. FP+AP i.d./MVA+AP i.d.
8. PBS x 2

Claims

1) A vaccine for inducing an immune response to an antigen, the vaccine comprising the antigen and a vector, the vector in the absence of the antigen inducing a weak or negligible antibody response and in the presence of the antigen inducing a T cell response complementing the antibody response against said antigen, the vector and the antigen being formulated separately or together.

2) A vaccine according to claim 1, wherein the vector is a poxvirus.

3) A vaccine according to claims 1 or 2, wherein the vaccine is suitable for administration in a homologous prime boost vaccination regimen.

4) A vaccine according to claims 1 or 2, wherein the vaccine is suitable for administration in a heterologous prime boost vaccination regimen.

5) A vaccine according to any preceding claim, wherein the antigen and the viral vector are admixed and administered as a mixture.

6) A vaccine according to any claim 1, wherein the viral vector is an adenovirus.

7) A vaccine according to any claims 1-5, wherein the vector is MVA, NYVAC, ALVAC or a fowlpox virus.

8) A vaccine according to any claims 1-5, wherein the vector is not ALVAC.

9) A vaccine according to any preceding claim that induces both an effector T cell response and an antibody response, wherein the effector T cell response is not weaker than that induced by the viral vector alone, and the levels of antibody induced are not lower than those induced by administration of the antigen alone.

10) A vaccine according to any preceding claim, wherein the antigen is derived from *M. tuberculosis*, *Plasmodium sp*, influenza virus, HIV, Hepatitis C virus, Cytomegalovirus, Human papilloma virus, bacteria, *Plasmodium sp*, leishmania parasites or is derived from a tumour.

11) A vaccine according to any claim 10, wherein the bacteria are Mycobacteria.

12) A method for stimulating both humoral and antibody responses to an antigen, comprising administration of the antigen to a patient in combination with a viral vector, administration of the vector and antigen being separately or together.

13) A vaccine according to any preceding claim, wherein the antibody response to the co-administered antigen is greater than the antibody response induced by the administration of a vaccine comprising said antigen and alum, but without the vector.

14) A vaccine according to any preceding claim, wherein the vaccine comprises alum, co-administered with the vector.

15) A vaccine according to claim 2, wherein the poxviral vector is an orthopox virus, such as MVA or NYVAC, the presence of an orthopox viral vector inducing substantially equal ratios of Th-1 and Th-2 Helper T cells.

16) A vaccine according to claim 2, wherein the poxviral vector is an avipox virus, such as Fowlpox or Canarypox, the presence of an avipox viral vector inducing a T cell mediated response, wherein the Th-1 Helper T cell response is greater than the Th-2 response.

17) A vaccine, comprising a polynucleotide and a co-administered antigen, the polynucleotide inducing a T cell response and the co-administered antigen inducing an antibody response, both the T cell and antibody responses being directed to said antigen;

wherein administration of the vaccine comprising the polynucleotide but not the co-administered antigen induces a weak or negligible antibody response;

the polynucleotide and the co-administered antigen being formulated separately or together, being further formulated for co-administration,

the Th-2 Helper T cell-mediated response being greater than the Th-1 response.

18) A vaccine, comprising a first antigen and a viral vector, formulated separately or together, wherein the antigen and the vector are formulated for co-administration.

19) A vaccine according to claim 1 wherein the vector and the antigen are formulated for co-administration.

20) A vaccine according to any preceding claim, wherein the co-administered antigen is not a polynucleic acid.

21) A vaccination method comprising co-administering an antigen together with a vector,
the method inducing both
- an antigen-specific T cell response to a poxvirus-encoded antigen, the encoded antigen being heterologous to the poxvirus and comprising a source of $CD4^+$ and $CD8^+$ epitopes; and
- antibodies to the co-administered antigen.

22) A method according to claim 21, wherein the co-administered antigen is not a polynucleic acid.

23) A method of inducing antigen-specific T cell responses in a vertebrate to a poxvirus-encoded heterologous polypeptide antigen comprising a source of $CD4^+$ and $CD8^+$ epitopes for the vaccinee and inducing antibodies to a co-administered (non-encoded) non-nucleic acid antigen by co-administration of the non-encoded antigen mixed with the said poxvirus.

24) A method of generating an antibody response to an antigen in a vertebrate vaccinee by co-administration, as a mixture, the antigen mixed with an orthopox virus.

25) The method of claim 24 wherein the orthopox virus is replication-impaired.

26) The method of claim 24 wherein the orthopox virus is of the modified vaccinia virus Ankara strain or NYVAC strain or a derivative of either.

27) The method of claims 24-26 wherein the orthopox virus encodes the co-administered antigen or a homologous sequence.

28) The method of claims 24-26 wherein the orthopox virus encodes an antigen that is heterologous to the co-administered antigen.

29) The method according the claims 24-26 wherein the vaccinee is a primate.

30) The method according to claim 29, wherein the vaccinee is a human.

31) The method according to claims 25-30 wherein the orthopox virus encodes a heterologous polypeptide antigen encoding a CD4+ and / or CD8+ T cell epitope against which the vaccinee has a pre-existing specific cellular immune response that was generated by a means other than by immunization with the said recombinant orthopox virus.

Abstract

Vaccination comprising co-administration of an antigen and a viral vector provides immunity at both humoral and antibody levels.